

Epidemiology and control of polioviruses in Nigeria

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Dedicated to Eve

Abstract

Although the World Health Assembly resolved to eradicate wild poliovirus (WPV) by 2000, four countries have yet to interrupt transmission of WPVs. As of 2009, Nigeria has reported the largest burden of poliovirus of any single country for 5 of the last 6 years and been responsible for the largest outbreak of circulating vaccine-derived poliovirus (cVDPV) to date. With surveillance data on 36,410 acute flaccid paralysis cases from 2001-2009 in Nigeria, I use case-control methods to estimate vaccine efficacies of trivalent and monovalent oral polio vaccines (tOPV and mOPVs, respectively) against paralysis by all types of poliovirus circulating in Nigeria (serotypes 1 and 3 WPV and serotype 2 cVDPV (cVDPV2)). I show that mOPVs are substantially more efficacious than tOPV against serotypes 1 and 3 and that tOPV is substantially more effective against cVDPV2 than against serotypes 1 and 3 WPVs. I use these results to investigate temporal and spatial variation in vaccine-induced immunity. I also estimate vaccine coverage and thus demonstrate that polioviruses persist in northern Nigeria as a result of low vaccine coverage and not due to any geographical variation in vaccine efficacy. I show that the clinical characteristics and attack rate of cVDPV2 are as would be expected of a WPV and therefore similar control methods should be used. Using a proportional hazards model, I demonstrate that the risk of reporting cVDPV2 is strongly localised and cVDPV2 can be controlled with well targeted use of tOPV whilst maintaining control of WPVs with mOPVs. Finally I use negative binomial regression to estimate the predictors of incidence by geographical state in Nigeria. I conclude that the prospects for elimination of all polioviruses in Nigeria are very good with targeted use of vaccines of the appropriate serotype and provided vaccine coverage can be improved in high incidence areas.

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Table of abbreviations

AFP	Acute Flaccid Paralysis
aVDPV	Ambiguous Vaccine-Derived Poliovirus
bOPV	Bivalent Oral Polio Vaccine (against types 1 and 3)
CCID	Cell Culture Infectious Doses
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
cVDPV	Circulating Vaccine-Derived Poliovirus
cVDPV2	Circulating Vaccine-Derived Poliovirus type 2
DfID	Department for International Development
DHS	Demographic Health Survey
GCC	Global Commission for the Certification of the Eradication of Poliomyelitis
GPEI	Global Polio Eradication Initiative
IPD	Immunisation Plus Day
IPV	Inactivated Polio Vaccine
iVDPV	Immunodeficiency Vaccine-Derived Poliovirus
LGA	Local Government Area
mOPV	Monovalent Oral Polio Vaccine
mOPV1	Monovalent Oral Polio Vaccine against Type 1 Polio
mOPV3	Monovalent Oral Polio Vaccine against Type 3 Polio
NICS	National Immunisation Coverage Survey
NID	National Immunisation Day
OPV	Oral Polio Vaccine
R₀	Basic reproduction number
SIA	Supplementary Immunisation Activity
sNID	Sub-National Immunisation Day

tOPV	Trivalent Oral Polio Vaccine
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
VAPP	Vaccine Associated Paralytic Poliomyelitis
VDPV	Vaccine-Derived Poliovirus
WPV	Wild poliovirus
WPV1	Wild poliovirus type 1
WPV2	Wild poliovirus type 2
WPV3	Wild Poliovirus type 3
WHO	World Health Organization

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Declaration of originality

Chapters 1 and 7 (Introduction and Discussion) were written by Helen Jenkins with comments from Christl Donnelly and Nicholas Grassly.

Chapter 3: The case control study used for vaccine efficacy analysis was conceived by Nicholas Grassly. Analyses in section 3.3.3 (Estimation of vaccine-induced population immunity and resulting confidence intervals in Chapter 5) were conceived and carried out by Jukka Corander and have been included in this thesis for completeness. Analyses in section 3.4 (Sensitivity of testing for poliovirus among Acute Flaccid Paralysis cases (Chapter 4)) use a method conceived and published by Gary et al. in 1997. Analyses in sections 3.7 (Estimation of hazard of reporting a cVDPV2 case (Chapter 5)) and 3.8 (Prediction of incidence of paralysis due to wild poliovirus type 1 (Chapter 6)) were conceived by Helen Jenkins. All other analyses in Chapter 3 were conceived jointly by Helen Jenkins, Nicholas Grassly, Christl Donnelly and Bruce Aylward. All analyses (other than those in 3.3.3 as mentioned above) were carried out by Helen Jenkins under the supervision of Nicholas Grassly and Christl Donnelly. Chapter 3 (apart from section 3.8) combines the methods sections of the papers that have been published from this thesis (Chapters 4 and 5) and was drafted by Helen Jenkins with input from Nicholas Grassly and Christl Donnelly. Section 3.8 was written by Helen Jenkins with comments from Nicholas Grassly and Christl Donnelly.

Chapters 4 and 5 are virtually identical to the published papers. These papers were drafted by Helen Jenkins with input from Nicholas Grassly, Christl Donnelly and Bruce Aylward.

Chapter 6 was written by Helen Jenkins with comments from Christl Donnelly and Nicholas Grassly.

All data on poliovirus were collected and approved by Alex Gasasira, Michael Mwanza, Sandra Garnier, Claire Chauvin, Emmanuel Abanida and Muhammad Ali Pate. Laboratory work was conducted in Nigeria directed by Festus Adu and Marycelin Baba.

1. Introduction

1.1 Poliomyelitis

Poliomyelitis (polio) is an infectious disease caused by poliovirus, one of the enterovirus group¹. There are three serotypes of poliovirus (called 1, 2 and 3) with each serotype conferring lifelong immunity to the host of that specific serotype². Transmission can occur via the fecal-oral route or nasopharyngeal routes and humans are the only known natural host. The clinical marker for polio is Acute Flaccid Paralysis (AFP) which is characterized by the rapid onset of weakness usually in one or more limbs progressing to maximum severity within several days to weeks with the absence of spasticity³, which occurs in less than 1% of cases (all other infections are asymptomatic or cause flu-like symptoms) and can cause death if the breathing muscles are affected.

While an Egyptian stele of a priest with a withered leg is suggestive of polio in humans for thousands of years², annual outbreaks only began to cause real concern at a public health level in the first part of the 20th century⁴. By the 1940s, several thousand children each year would become infected and paralysed by polio in the United States of America alone and huge resources were directed at learning more about the epidemiology of polio and discovery of a vaccine (see 1.3). Following a lengthy eradication programme (see 1.5) polio is now limited to a handful of countries and causes around 1,500 paralysis cases each year². We are on the brink of eradicating an infectious disease for the second time in history (smallpox was declared eradicated by the World Health Organization (WHO) in 1979) but important questions remain over the feasibility of this objective.

1.2 Epidemiology of polio

Poliovirus can implant and replicate in the throat or intestines and then be shed in the feces or nasal and throat passages⁵. Shedding usually begins from 3 days after exposure⁵. Virus is generally shed in the feces

for on average 6-7 weeks^{6,7} and for up to 3-4 weeks from the nasopharynx⁸. The incubation time for paralytic polio from exposure to onset of paralysis is estimated to be 16.5 days (standard deviation 5.2 days)^{7,9}. Due to excretion times, transmission is more common via the fecal-oral route rather than nasopharyngeal and hence becomes more likely in countries with poor sanitation where the fecal-oral route is more common as compared to countries with effective sanitation where the nasopharyngeal route is usual⁸.

The paralysis case to infection case ratio is difficult to estimate accurately, complicated by the impact of vaccination and studies which have tried to provide estimates have often been small. Gelfand et al.¹⁰ showed, in a study of 105 infected children in 1954/5, that infection with each serotype was equally likely but that paralysis was 5 times as likely when infected with serotypes 1 and 3 than serotype 2. These data were obtained from isolation of poliovirus from stool samples of children sampled on a monthly basis in Louisiana and then examination of which infections had resulted in paralysis. Fox et al.¹¹, using data from the same Louisiana study, estimated that the case to infection ratio was 1:850 in the under 30 year old population however this did not distinguish between serotype. This estimate was obtained using the same data as above and extrapolating to the whole population using the number of paralysis cases observed. Penttinen and Patiala¹² conducted a study in Finland taking blood from individuals aged 6-12 years old and testing sera for polio antibodies. They found that the case to infection ratio for serotype 1 polio was 1:150. In 1948, in a study in North Carolina, Melnick and Ledinko¹³ estimated the age-specific ratio of paralytic cases to inapparent infections for serotype 1 polio to be 1:175 for age 0-1 years, 1:86 for 1-4 years, 1:62 for 5-9 years and 1:95 for 10-14 years. Again, individuals in this study were bled and their blood tested for polio antibodies. A number of studies were reviewed by Nathanson and Martin⁴ which gave estimates of paralytic cases per 100 infections from <0.1 to 4.5. An earlier estimate of 1:1000 was obtained by analysis of sewage in Manhattan, New York and estimation of the number of poliovirus carriers in the population from the amount of poliovirus found in the sewage¹⁴. Although estimates vary,

the case to infection ratio is generally quoted as around 1:200 for serotype 1 and around 1:1000 for serotypes 2 and 3¹⁵, although in the presence of mass vaccination these may be smaller (see 1.3).

Where polio outbreaks have been studied in fully susceptible populations, it appears that young adults are the most likely age group to become paralysed even though attack rates (in terms of infection) are similar throughout all ages, although this trend can vary in populations that are partially immune¹⁶. Males are more likely to become paralysed at younger ages than females and in older age groups, the reverse is true^{17, 18}.

Polio epidemics appear to exhibit seasonality which is more marked further away from the equator and less apparent in the tropics¹⁹.

1.3 Vaccination against polio

There is currently no cure for polio although two types of vaccine exist. However, research has recently investigated the possibility of antivirals for polio. In 2005, the National Academy of Sciences hosted a workshop on antiviral compounds and work is continuing although with limited funding². It is hypothesized that polio antivirals could be of use in an outbreak situation, particularly if used in conjunction with the inactivated polio vaccine (see below). Antivirals have short-term effects and therefore are inferior to the long-term impact of a vaccine, however, in an outbreak they could reduce viral replication and shedding in infected people to limit transmission while sufficient numbers of vaccine doses are administered (for example, 2 doses at least 1 month apart)²⁰. They could also be useful in the event of accidental release to limit viral shedding in workers that would have already received the inactivated polio vaccine but could still potentially shed virus (see below)²⁰.

The inactivated (killed) polio vaccine (IPV), protecting against all three poliovirus serotypes was developed in 1955 by Jonas Salk and colleagues. IPV works by producing antibodies in the blood inducing humoral immunity which prevent the poliovirus from reaching the central nervous system²¹. Therefore, IPV does not necessarily prevent infection with poliovirus but does prevent the potential resulting paralysis. There is however evidence that immunization with IPV can reduce excretion of poliovirus from the nasopharynx²² thus reducing the probability of transmission via this route although in areas of poor sanitation where the fecal-oral route is more common, IPV may not be sufficient to prevent transmission. IPV must be injected and therefore requires administration by a trained health worker. This also potentially makes IPV unsuitable for mass vaccination campaigns where large numbers of children are vaccinated in a short timeframe.

The oral polio vaccine (OPV) was developed in 1961 by Albert Sabin and colleagues and has been the most widely used vaccine for reasons of cost effectiveness, ease of oral administration and the secondary protection that is sometimes conferred to contacts of the recipient²³. Additionally, OPV works in two ways to protect recipients against polio. Like IPV, it produces antibodies in the blood which induce humoral immunity and prevent the virus from reaching the central nervous system where they could cause paralysis. In addition, OPV produces an immune response in the lining of the intestines and throat (“local” or “mucosal” immunity) preventing the virus from multiplying and hence protecting against infection²⁴. This mucosal immunity can effectively prevent onward transmission of poliovirus by the fecal-oral route – likely to be the most common route in settings with poor sanitation. However since it uses a live vaccine virus and as a consequence of genetic instability of the OPV strains, it carries a risk of causing vaccine associated paralytic polio (VAPP) in the recipient. In the USA, the probability of this is estimated at 1 in 750,000 doses following the first dose, dropping to around 1 in 5 million doses for subsequent doses²⁵. In the early 1960s, monovalent OPVs (mOPVs) which each protected against just one serotype were used extensively. After 1963, mOPVs were replaced in most countries with trivalent OPV (tOPV), which contains all three poliovirus serotype strains, for reasons of logistical simplicity²⁶. While

tOPV has successfully eradicated polio in most of the world, interference between the three vaccine virus serotypes during infection and replication in the gut can reduce the efficacy¹ of the component serotypes, especially serotypes 1 and 3²⁷. This becomes a challenge in developing country settings where additional factors such as infection with other pathogens or diarrhea can further reduce the efficacy of OPV^{28, 29}.

Therefore, in 2004, following reviews of mOPVs used in the early 1960s and in a small number of countries since, the Advisory Committee on Polio Eradication recommended the use of mOPV against serotype 1 polio (mOPV1)²⁶, which was first used in India in 2005³⁰ and Nigeria in 2006³¹. mOPV1 has since been rolled out to other endemic countries and those suffering from imported cases and mOPV against serotype 3 polio (mOPV3) has also been licensed and used in polio infected countries.

Additionally mucosal immunity following vaccination with OPV may vary by serotype, location and vaccine formulation allowing some children to become infected and transmit virus while still protecting them from paralysis^{32, 33}.

Several countries switched from OPV to IPV once the risk of VAPP exceeded that of contracting wild poliovirus^{25, 34} although these were largely industrialized countries in which polio transmission would be less likely to occur via the fecal-oral route. The risk of polio being transmitted widely if OPV were to be replaced with IPV in countries with poor sanitation is unknown and potentially significant³⁵. Some research has been done and continues to be carried out examining the possibilities of combined schedules using both IPV and OPV. In 2000, a trial in Oman showed that a supplementary dose of IPV following 5 previously administered doses of OPV increased serotype 3 prevalence from 87.8% to 97.1% and also increased the median antibody titer whereas a supplementary dose of OPV had no such effect³⁶.

Simultaneous use of OPV and IPV to induce mucosal immunity is one research area of interest to the Global Polio Eradication Initiative (GPEI – see below)³⁷.

¹ Note that throughout this thesis, the term ‘efficacy’ is used to describe the field efficacy of OPVs

1.4 Vaccine-derived polioviruses

In addition to the risk of VAPP, each dose of OPV also has a probability of mutating or recombining with other enteroviruses to become a vaccine-derived poliovirus (VDPV). VDPVs are defined as polioviruses that differ from the parental vaccine strain at 1% to 15% of VP1 nucleotides²¹. Any virus that differs by more than this is classed as a wild poliovirus and anything less, as an OPV-like isolate.

There are three main classifications of VDPV. Sometimes, an OPV-isolate that has recombined with another enterovirus or mutated to become a VDPV regains the potential to be transmissible as a circulating VDPV (cVDPV) and an outbreak of cVDPV is defined as more than 1 paralytic case caused by the same strain². As of August 2009, there have been 12 known geographically isolated outbreaks of cVDPV, 11 since 2001 which have been identified from surveillance data and samples while they were circulating in a population^{2, 38} and 1 in Egypt in the 1980s and 90s which was only identified by retrospective analysis of samples³⁹. While three outbreaks are still ongoing as of October 2009 (in Nigeria, DR Congo and Ethiopia), the other eight appear to have been stopped through increased vaccination campaigns³⁸. Many of the outbreaks have been associated with low population immunity to poliovirus through a combination of low natural immunity resulting from elimination of wild poliovirus in the area and low vaccination coverage^{21, 38, 40}. In reality, a cVDPV probably requires a trade-off between a level of vaccination coverage which will allow for sufficient doses to be administered to increase the probability of an OPV-isolate reverting to wild poliovirus transmissibility but also sufficient numbers of children left unimmunized so as to enlarge the susceptible pool that can be infected once the initial reversion has occurred. There has been some debate over how dangerous cVDPVs are and whether they should be considered in the same way as wild polioviruses⁴¹. It has been suggested that cVDPVs may be less likely to cause paralysis and may be less transmissible than wild polioviruses and hence may be easier to control⁴².

Immunodeficiency VDPVs (iVDPVs) have occurred when there has been prolonged intestinal replication of OPV viruses in people with rare immune deficiency disorders²¹. There have only been 23 documented cases of this to date⁴³ and so far, no evidence of onward transmission⁴⁴. Ambiguous VDPVs (aVDPVs) are those isolated from patients without any immunodeficiency disorder unrelated to any outbreak or from environmental samples that cannot be linked to any other source²¹. Only 16 aVDPVs were detected from 1965 to 2004 although there is a suspicion that some of these may have been iVDPVs or cVDPVs (see below)²¹.

1.5 Polio eradication

In 1988, the World Health Assembly, consisting of the Ministers of Health of all member states, unanimously passed a resolution to eradicate² polio by 2000⁴⁵. The Global Polio Eradication Initiative (GPEI) was to be led by the World Health Organization (WHO), Rotary International, Centers for Disease Control and Prevention (CDC) and the United Nations Children's Fund (UNICEF) through improved routine immunization and use of SIAs (Supplementary Immunisation Activities using OPV). SIAs became necessary in areas where routine immunization coverage was low and/or vaccine efficacy was low. They usually take the form of mass vaccination campaigns where voluntary vaccinators go from village to village or even door to door vaccinating all children under 5 years old. Sometimes these will take place over 2 or 3 days co-ordinated across an entire country (National Immunisation Days or NIDS) or across part of a country (sub-NIDS or sNIDS) and sometimes they are in the form of a "mop-up" campaign where an area is targeted in reaction to a recently reported case. Eradication is considered feasible given that no animal reservoir exists and the disease is vaccine preventable. In 1988, over 125 countries had endemic polio with an estimated 350,000 worldwide paralysis cases. In 1999, the last case of wild poliovirus serotype 2 was reported⁴⁶ and three WHO regions (the Americas, the Western Pacific and

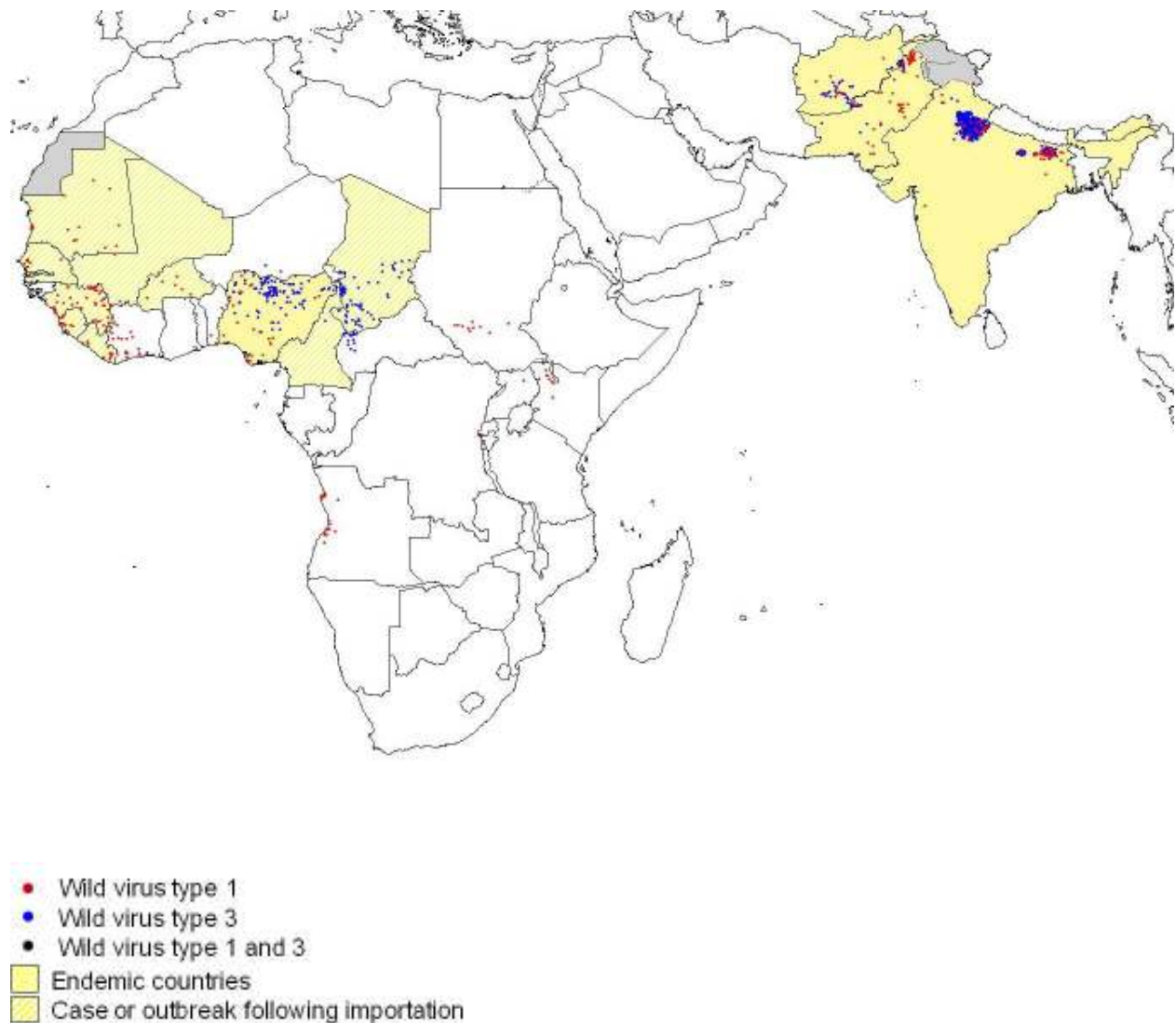
² Disease is eradicated from the entire world but eliminated from a country or region

Europe) have been declared polio-free (i.e. the transmission of wild poliovirus has been interrupted and no cases have been reported for at least 3 years)⁴⁷. Elimination in these regions and eradication of serotype 2 are seen as proof of principle that all serotypes can be eradicated. In 2007, four countries had still never interrupted transmission of serotypes 1 and 3 successfully – India, Nigeria, Pakistan and Afghanistan – and 1312 paralysis cases were reported worldwide². Polio eradication is often compared to that of smallpox and this highlights some of the difficulties. The OPV has low efficacy in some settings and requires at least 3, if not up to 8 or more doses to successfully immunize a child; the small pox vaccine required only one dose. Since the majority of polio infections are asymptomatic or cause symptoms easily confused with other illnesses, polio can spread silently through a community whereas small pox had clearly defined symptoms allowing for effective ring vaccination when a case was detected. Finally, the global population was around 4 billion during the time of small pox eradication; now at nearly 7 billion, with increased population density and travel, polio transmission is easier and control and vaccination are harder and more expensive.

Polio elimination has however been achieved in the majority of the world and the reasons for failure to eliminate polio in the four remaining pockets vary. Therefore, the GPEI has had to examine the epidemiology of the disease in each country and tailor strategies accordingly. In India, poor sanitation (facilitating transmission) and high incidence of diarrhea (not allowing the vaccine to confer immunity) have been shown to be associated with incidence of polio, despite some children having received 15 or more doses of OPV⁷. Additionally, tOPV has been shown to be less efficacious against serotypes 1 and 3 polio as it is against serotype 2 due to interference between the three vaccine strains²⁷. Therefore, with these factors in mind and recognition that tOPV alone may not eradicate polio, mOPV1 was licensed in India. In 2007, the increased efficacy in India of mOPV1 in comparison with tOPV was confirmed to be 3-fold⁴⁸ in line with other studies of the efficacy of mOPV1⁴⁹. Problems in Afghanistan and Pakistan have been exacerbated by conflict and instability across the region resulting in large-scale population movements which aid polio transmission and limit the number of children receiving regular, if any,

vaccination rounds. Solutions such as cross border co-ordination of SIAs, agreements with the Taliban and so-called “Days of Tranquility” have helped the eradication effort but elimination in these two countries is yet to be achieved⁵⁰. See below for details on the situation in Nigeria.

Figure 1.1 Map of reported wild polio cases with date of onset between 31 March 2009 and 30 March 2010 [Source: Global Polio Eradication Initiative²]



Good quality surveillance for polio cases is key to the eradication strategy. This process and the data that it produces are described below (1.7).

Although OPV may be able to eradicate wild polioviruses, it will always have the potential to cause VDPVs and therefore polio could never be declared completely eradicated while OPV is in use. Therefore, part of the eradication program has also focused on the era post eradication of wild polioviruses including a global cessation of OPV use three years after the last wild poliovirus case is reported⁵¹. There is considerable debate about the feasibility of polio eradication and cessation of OPV use^{42, 52-54}. Several countries switched from OPV to IPV once the risk of VAPP exceeded that of contracting wild poliovirus^{25, 34}, however in the post eradication era, this decision will be made by individual countries. In reality, many countries will not be able to afford vaccination with IPV and therefore the susceptible population will grow rapidly – a potential problem in the event of laboratory escape or bioterrorism. Research is currently being carried out on alternative vaccination options such as needle-free IPV, which will allow for lower dosage, reduce costs and make administration of the vaccine considerably easier and Sabin IPV which will allow production in developing countries⁵⁵.

1.6 The polio vaccination boycott in Nigeria

The inability to eliminate polio from Nigeria appears to stem from a polio vaccine boycott. In 2003, some religious leaders in Kano state in Northern Nigeria, in the belief that OPV was contaminated with sterilising agents, ordered the temporary suspension of all polio immunization in Kano⁵⁶. This contributed to a national epidemic of polio and the re-infection of at least 20 previously polio-free countries^{57, 58}.

Although polio immunization was reinstituted within 12 months, coverage remained low across Northern Nigeria and case numbers continued to increase. The reasons behind continuing low uptake of the polio vaccine however go beyond the original boycott. In 2005, Elisha Renne carried out a study interviewing

residents of Zaria town in Kaduna state in Northern Nigeria⁵⁹ asking for their perspectives on polio and immunization. She found continuing skepticism following the boycott, not only because of trust that people had in their local leaders who had halted the immunization campaigns, but also for other reasons. Some were suspicious of a vaccine that was free and was being brought direct to their homes while other drugs were charged for or required a visit to the hospital. This suspicion was further accentuated among people who knew that OPV could cause paralysis and that IPV was being used in Western countries. Others were confused by the availability of polio vaccines when polio was causing relatively few deaths in comparison with measles and malaria for which vaccines and medications seemed in shorter supply. Renne also cited a breakdown in the provision of routine services. Four doses of OPV are given at birth, 6, 10 and 14 weeks in Nigeria as part of the routine immunization programme but OPV rates are particularly low in Northern Nigeria, in some states less than 20% of children receive the recommended 3 doses by one year of age⁶⁰.

Ayodele Jegede⁶¹, writing in 2007, also pointed out that utilization rates of orthodox healthcare services in Northern Nigeria have historically been lower than in the South underlying a general mistrust of modern medicine and strong belief in local Muslim clerics and traditional chiefs and medicines. He also described how an attempt at population control in the 1980s was connected, in the view of some people, with immunization and there is still a belief that the government may use vaccination as a way of controlling the population. Finally, Pfizer's "Trovan trial" in Northern Nigeria in 1996 in which five children died after allegedly being given an "untested, unproven drug without first obtaining their informed consent"⁶² has only exacerbated mistrust of Western medicine^{63, 64}.

There have been very few data based studies on polio in Nigeria. In 2005, Adewumi et al. took blood samples from 582 children in South West Nigeria and looked at neutralizing antibody titre to the three poliovirus serotypes⁶⁵. They found that 59.6% of children had at least antibody titre of 1:8 against each of the three poliovirus serotypes. They also found that more children had titre of at least 1:8 against serotype

2 than serotypes 1 and 3 and that immunity increased with age. The authors concluded that a high number of children were left at risk from infection with one or more poliovirus serotypes in a country with high polio incidence. A study in 1997 by Odemuyiwa et al. looked at cord blood from 72 newborns and found that some had no antibodies to poliovirus serotypes 1 and 3 (3% and 28% respectively)⁶⁶. The authors concluded that maternal antibodies cannot be relied upon for protection against polio and stress that importance of a birth dose of OPV. In 1996, Adu et al examined fecal samples from 146 children in Ibadan and found 8 strains of wild poliovirus serotype 1: 3 from unvaccinated infants and 5 from fully vaccinated children confirming circulation of wild poliovirus serotype 1 even amongst vaccinated children⁶⁷.

1.7 Surveillance for polio cases and data used in this thesis

As mentioned in 1.5, surveillance for polio requires detecting all cases of AFP quickly and assessing them for presence of poliovirus in stool samples. In each country, this requires a good network of communication so that once a parent/carer has reported paralysis in a child to the local health worker (of whichever type, in some rural areas this may be someone working as a religious or traditional healer) this information must be reported without delay to a more central health authority so that WHO investigators can be sent in to interview the parent/carer, examine the child and take stool samples. AFP can be caused by a number of conditions other than polio including Gullain-Barré syndrome and infection with other enteroviruses³. In 1995, the Global Commission for the Certification of the Eradication of Poliomyelitis (GCC) determined the criteria for certification including the definition of certification standard surveillance⁶⁸. Under these criteria, the required standard for reporting of AFP is at least 1 case reported annually per 100,000 of the population aged under 15 years⁶⁹. The guidelines for stool sample collection require that two stool samples are collected 24-48 hours apart and within 14 days of onset of paralysis⁶⁹. They are then sent to within country laboratories (which must be WHO-accredited⁶⁹) and assessed for

presence of polioviruses. Countries should be aiming to collect and analyse stool samples in this way for at least 80% of AFP cases^{69, 70}. Figure 1.2 shows recent achievement of these two objectives by country.

Figure 1.2 AFP surveillance objectives, (a) rate of reporting of non-polio AFP per 100,000 in the population for the period March 2009- February 2010, (b) adequate stool collection rate for the period February 2009- January 2010 (2 samples between 24-48 hours apart within 14 days of onset of paralysis)
[Source: Global Polio Eradication Initiative²]

(a)



(b)



As a result of this surveillance, data are available on all reported AFP cases throughout Nigeria from the investigations that take place once the AFP is reported. A case investigation form is completed prior to analysis of samples and hence without knowledge of the polio status of the child. Information collected includes date of birth, date of onset of paralysis, location of residence (Local Government Area (LGA) which is also known as district, of which Nigeria has 774), sex, number of OPV doses received and clinical history (for example, presence of fever at onset of paralysis and site of paralysis). Following analysis of stool samples, results are communicated back to the patient and relevant local medical practitioners and the laboratory results are added to this database. This is the main data source used in this thesis and provides information on, not only all paralytic polio cases but also non-polio AFP cases that can be used as a control group where necessary.

Additional to this, the National Immunisation Coverage Survey (NICS)⁶⁰ has been a useful source of data on routine immunization rates by state in Nigeria.

1.8 Thesis aims and structure

This thesis aims to investigate the epidemiology and control of polioviruses in Nigeria through biostatistical analysis of the surveillance data described in 1.7. Chapter 2 gives further details of the data used in this thesis. Some methods are applicable to more than one chapter, therefore to prevent repetition Chapter 3 describes all methods used throughout the thesis. Since this PhD began, in 2007, the situation with respect to polio in Nigeria has evolved. Focus was placed on elimination of wild poliovirus serotype 1 in 2007 and incidence dropped markedly raising expectations of imminent elimination of wild poliovirus serotype 1 in Nigeria. Chapter 4 examines whether low vaccine coverage was genuinely the reason for polio persistence and how this had changed leading up to 2007. It also evaluates mOPV1, introduced in Nigeria in 2006 and other interventions used by the GPEI. This background is described in more detail in the introduction to Chapter 4 and the results presented and their implications are also

discussed in this chapter. In 2008 a large outbreak of wild poliovirus serotype 1 occurred spreading to Southern states and several other African countries. In 2009, wild poliovirus serotype 3 incidence has increased and wild poliovirus serotype 1 incidence has declined again. Meanwhile, a serotype 2 cVDPV (cVDPV2) which was first detected in Northern Nigeria in 2005 and had been causing a low level of morbidity in the Northern states has spread south and caused twice as many cases in the first six months of 2009 as compared to the whole of 2008. In Chapter 5, this cVDPV2 is examined in detail and compared with the two circulating wild polioviruses in Nigeria. The efficacy of tOPV against this cVDPV2 is estimated and the clinical and epidemiological characteristics of cVDPV2 cases are compared with those of wild polioviruses in the same setting. The risk of the cVDPV2 spreading to new areas is estimated using a proportional hazards model with time-varying covariates. Again, a fuller introduction to this is given in Chapter 5 along with results and a discussion. In 2008/9, several other African countries were re-infected with wild poliovirus serotype 1 originating in Nigeria. In Chapter 6, predictors of wild poliovirus serotype 1 incidence in Nigeria are assessed using a negative binomial regression model. Finally, all results, their implications and the future of polio eradication in Nigeria are discussed in Chapter 7.

2. Data sources and description

2.1 Data sources

Cases of poliomyelitis were identified from the national database maintained by the Government of Nigeria that has involved detection and reporting of all cases of AFP among children aged under 15 years since December 1996⁷¹. AFP is characterized by the rapid onset of weakness progressing to maximum severity within several days to weeks with the absence of spasticity or other signs of disordered central nervous system motor tracts such as hyperreflexia, clonus or extensor plantar responses³. Since 2001 national surveillance quality has exceeded the international performance standard of at least 1 annual case of non-polio AFP reported annually per 100,000 children⁶⁹.

Whenever a case of apparent AFP is reported to local health services, it is reported to the GPEI staff in Abuja and a Disease Surveillance and Notification Officer (DSNO) conducts a case investigation as soon as possible. The data collected on initial interview in the Case Investigation Form are listed in Table 2.1. The Case Investigation Form itself can be seen in Appendix 1.

Table 2.1 Variables collected in the Case Investigation Form. Those in bold are collected at the initial investigation. Those relating to the stool samples are entered when the stool samples have been collected and analysed. The remaining variables are entered at the follow up examination.

Variable	Response options
ID number	Written out fully
LGA**	Written out fully
State**	Written out fully
Nearest health facility to village*	Written out fully
Address*	Written out fully

Village/neighbourhood*	Written out fully
Town/City*	Written out fully
Name of patient*	Written out fully
Father/mother*	Written out fully
Date of birth	dd/mm/yy
Age: years	Numerical
Age: months	Numerical
Sex	M=Male, F=Female
Notified by	Written out fully
Date notified	dd/mm/yy
Date case investigated	dd/mm/yy
Admitted to hospital	1=Yes, 2=No
Date of admission	dd/mm/yy
Medical record number	Numerical
Name/address of facility	Written out fully
Date onset of paralysis	dd/mm/yy
Fever at onset of paralysis	1=Yes, 2=No
Flaccid and sudden paralysis	1=Yes, 2=No
Paralysis progressed 0-3 days	1=Yes, 2=No
Asymmetrical	1=Yes, 2=No

Site of paralysis	A 2x2 grid indicating left and right arms and legs is completed with 1=Yes, 2=No for each box
After investigation, was this a true AFP?	1=Yes, 2=No
Total polio doses	Numerical, 99 if unknown
OPV doses: Birth dose	dd/mm/yy
OPV doses: 1st dose	dd/mm/yy
OPV doses: 2nd dose	dd/mm/yy
OPV doses: 3rd dose	dd/mm/yy
OPV doses: 4th dose	dd/mm/yy
OPV doses: If more than 4, last dose	dd/mm/yy
Date 1 st stool	dd/mm/yy
Date 2 nd stool	dd/mm/yy
Date stool specimen sent to national lab	dd/mm/yy
Date specimen received by national lab	dd/mm/yy
Condition of stool	1=Adequate, 2=Not adequate
Date result sent by lab to state	dd/mm/yy
Date result received by state	dd/mm/yy
Date isolate sent by national lab to regional lab	dd/mm/yy
Date differentiation result sent by regional lab	dd/mm/yy
Date differentiation result received by state	dd/mm/yy

Primary isolation result: P1 (Poliovirus serotype 1)	1=Yes, 2=No
Primary isolation result: P2 (Poliovirus serotype2)	1=Yes, 2=No
Primary isolation result: P3 (Poliovirus serotype 3)	1=Yes, 2=No
Primary isolation result: W1 (Wild poliovirus serotype 1)	1=Yes, 2=No
Primary isolation result: W2 (Wild poliovirus serotype 2)	1=Yes, 2=No
Primary isolation result: W3 (Wild poliovirus serotype 3)	1=Yes, 2=No
Primary isolation result: V1 (Vaccine virus serotype 1)	1=Yes, 2=No
Primary isolation result: V2 (Vaccine virus serotype 2)	1=Yes, 2=No
Primary isolation result: V3 (Vaccine virus serotype 3)	1=Yes, 2=No
Primary isolation result: NPENT (Non-polio enterovirus)	1=Yes, 2=No
Date follow-up examination	dd/mm/yy
Residual paralysis	A 2x2 grid indicating left and right arms and legs is completed with 1=Yes, 2=No for each box
Findings at follow up	1=Residual paralysis, 2=No residual paralysis, 3=Lost to follow-up, 4=Death before follow-up

Final classification of the case	1=Confirmed, 2=Compatible, 3=Discarded, 6=Not an AFP
Investigator name	Written out fully
Investigator title	Written out fully
Investigator unit	Written out fully
Investigator address	Written out fully
Investigator phone	Written out fully
Where has the child been in seeking help for this problem before presenting here (in sequence of visit)?	Place and duration written out fully

*Unavailable due to confidentiality

**Administratively, Nigeria is divided into 6 zones, 37 states and 774 Local Government Areas (LGAs or districts)

The Case Investigation Form is filled out manually and sent into the GPEI offices in Abuja where the data are entered into the surveillance database. All AFP cases undergo laboratory investigations at one of two laboratories in Nigeria including the collection of two stool samples, at least 24 hours apart, to test for poliovirus. Samples giving positive results are investigated by intratypic differentiation tests and genetic sequencing to determine whether the virus is vaccine-related or wild poliovirus⁷². A follow-up visit at 60 days includes testing for residual paralysis (see variables collected in Table 2.1).

These data are then sent, in the form of an Excel spreadsheet, to the WHO regional office (AFRO region) and then to WHO headquarters in Geneva. Confidential items of data are then removed from the database before it is sent to us at Imperial College, London.

The surveillance database supplied the majority of data that was used in this thesis. Additional data sources were used where necessary as described below.

Information on routine immunization was not available for individuals although overall rates by state were available. In 2006, the National Immunisation Coverage Survey (NICS)⁶⁰ was carried out and sponsored by the CDC, the WHO, UNICEF and the Department for International Development (DfID). This survey provides the percentage of infants that have received all 3 recommended doses of OPV by 52 weeks old. These rates were used throughout this thesis as described in more detail below. Rates of routine immunization coverage were also available in the Demographic Health Survey (DHS) 2003 as part of Measure DHS which is sponsored by the United States Agency for International Development (USAID). However, within Nigeria, the NICS is considered a more reliable source and indeed work carried out in Chapter 6 (see below) confirms that the NICS data provide a greater association with rates of reporting of wild poliovirus type 1 cases than data from the DHS.

2.2 Potential limitations and sources of error in the AFP surveillance database

There are errors in ascertaining the precise age of children at onset of paralysis. Where date of birth was available, this was used to calculate the age at onset of paralysis (by subtracting the date of birth from the date of onset of paralysis); date of birth was available for 56.6% of AFP cases. For some AFP cases, age in terms of years and/or months was completed. Where years and months were available, we assumed that the child's age was half way through the month and added 15 days (e.g. for a child that was 8 months old, that date of birth was calculated to be date of onset of paralysis – $((365/12)*8 + 15)$). Where only years were available, the same method was used assuming that the child's age was half way through that year (i.e. 183 days were added). Of all AFP cases, 12.3% of the ages were precise to the nearest month and 27.4% were precise to the nearest year. A further 3.7% of AFP cases were missing data on age entirely and had to be excluded from many of the analyses.

There were several potential errors in the OPV dose reporting. Firstly, there could be recall bias since parents/carers had to remember how many OPV doses children had received which can result in inaccuracies when remembering doses received several years ago. It is unclear whether this bias would

result in under- or over-estimates of OPV doses. Parents/carers may potentially worry that they will be accused of failing to protect their children against polio and so inflate the number of OPV doses their child has received. Conversely, there are reports (pers. comm. Mike Mwanza) of stigma associated with receiving OPV and the possibility that male heads of a household were more likely to say that the children had received zero doses. However, since data on OPV doses was collected before either the parents/carers or case investigators knew the polio status of the child, the comparison between cases and controls necessary for the vaccine efficacy analysis should be consistent regardless of the direction of the bias. Any consistent under or over reporting would result in under- or over-estimates of population immunity levels respectively. However, the comparisons over time and across the country would remain consistent. Secondly, only total OPV doses was collected and no individual level data on routine immunization doses. We therefore had to make certain assumptions about whether or not doses were received through routine services or SIAs. The ways in which this was done and how it was tested for are given in detail in Chapter 3.

As with any data collection where information is written down manually and then entered into a database, there is potential for human error. Checks for inconsistencies were carried out at the WHO Regional and level and WHO headquarters. When inconsistencies were found, they were sent back to the WHO office in Abuja where they were checked against the original (paper) information and, if necessary, the case was contacted in order to resolve the query. Due to the time consuming nature of the latter solution, focus was on the poliovirus cases and non-polio AFP cases were checked where possible. Sometimes further errors were found by cross checks at Imperial College with other variables in the dataset. For example, there was a small number of cases where the date of birth was later than the date of onset of paralysis ($<0.1\%$). In these cases either age in years/months was used (if it seemed appropriate) or the age was assumed to be missing. Some human error cannot be revealed with such cross checks but we can assume that these errors would not cause any specific bias in one direction or another across the data.

2.3 Definitions and exclusions

A case of type 1 or 3 poliomyelitis was defined as any case of AFP with virological confirmation of type 1 or 3 wild poliovirus (WPV1 and WPV3) respectively from at least one stool sample. We define a case of circulating vaccine-derived poliovirus ('cVDPV case') as any AFP case with vaccine poliovirus isolated from at least one stool sample that differed from the original Sabin vaccine strain by 1% to 15% of nucleotides in the region encoding the viral capsid protein VP1²¹. Specifically, a 'cVDPV2 case' is a serotype 2 cVDPV case. We define 'non-polio AFP cases' as those AFP cases where two adequate stool samples were collected within 2 weeks of the onset of paralysis, at least 24 hours apart and where neither wild nor vaccine poliovirus were isolated. Non-polio AFP is caused by a range of other conditions including Guillain-Barré syndrome and infection with other enteroviruses³. AFP cases without two adequate stool samples and residual paralysis (assessed at least 60 days after onset of paralysis) compatible with poliomyelitis were excluded from all analyses ("compatible cases"), as were cases with more than 1 wild poliovirus (WPV) serotype isolated (since we could not be sure which had caused the paralysis). Estimates of vaccine efficacy and population immunity additionally excluded AFP cases without reported number of OPV doses received or age at onset of paralysis (since those data are necessary for the estimations) and those excreting vaccine (Sabin) poliovirus (since there was a small possibility that their paralysis was a case of vaccine-associated paralytic polio (VAPP – see Introduction)).

2.4 Estimation of OPV products received by children in the AFP database

Routine immunization coverage in Nigeria is low; nationwide in 2006 just 37% of children had received three doses of tOPV by 52 weeks of age, but coverage is extremely heterogeneous dropping in northern states⁶⁰. Most polio vaccine doses are received through SIAs, introduced in 1996. The number of doses of each type of OPV received by children with AFP was therefore estimated based on their reported number

of OPV doses and the type of vaccine they were exposed to through SIAs only (calculated on the basis of their age and district of residence) (see Appendix Table A2 for the full list of SIAs conducted during the study period). The fraction of SIA that a child was exposed to that used each vaccine type was multiplied by the number of reported doses received to give an expected number of doses of each vaccine type received⁴⁸. The total number of mOPV doses was constrained so as not to exceed the number of SIA using monovalent vaccine and remaining doses were assumed to be tOPV. The effect of the limited routine coverage with tOPV recorded in each state on estimates of vaccine efficacy and population immunity was assessed on the basis of the NICS⁶⁰. The sensitivity of results to the method of calculating the type of vaccine received based on SIA only was assessed as described below in the statistical analysis section.

2.5 Potency and safety of vaccines

The potency of each newly licensed monovalent vaccine is equivalent to the individual components of the standard trivalent vaccine. Thus a dose of mOPV1 contains 10^6 median cell culture infectious doses (CCID₅₀) and a dose of mOPV3 contains $10^{5.8}$ CCID₅₀ as found in the trivalent formulation.

This work reports an analysis of a National Surveillance database recording use of standard vaccines licensed by the National Regulatory Authority of the Government of Nigeria for use in Nigeria.

Surveillance includes investigation of all potential cases of VAPP that typically occur following vaccination with OPV in approximately 1 child out of a million first dose recipients and at much lower levels for subsequent doses^{73, 74}.

2.6 Datasets used in each chapter and exclusions

While the AFP database described above is used throughout this thesis, each results chapter used a different subset of the full dataset. Figures 2.1, 2.2 and 2.3 describe the dataset used in each results chapter including the time period covered, number of observations and details of those excluded.

Figure 2.1 Flow chart illustrating the total number of AFP cases used in Chapter 4 (covers 1 January 2001 – 31 December 2007)

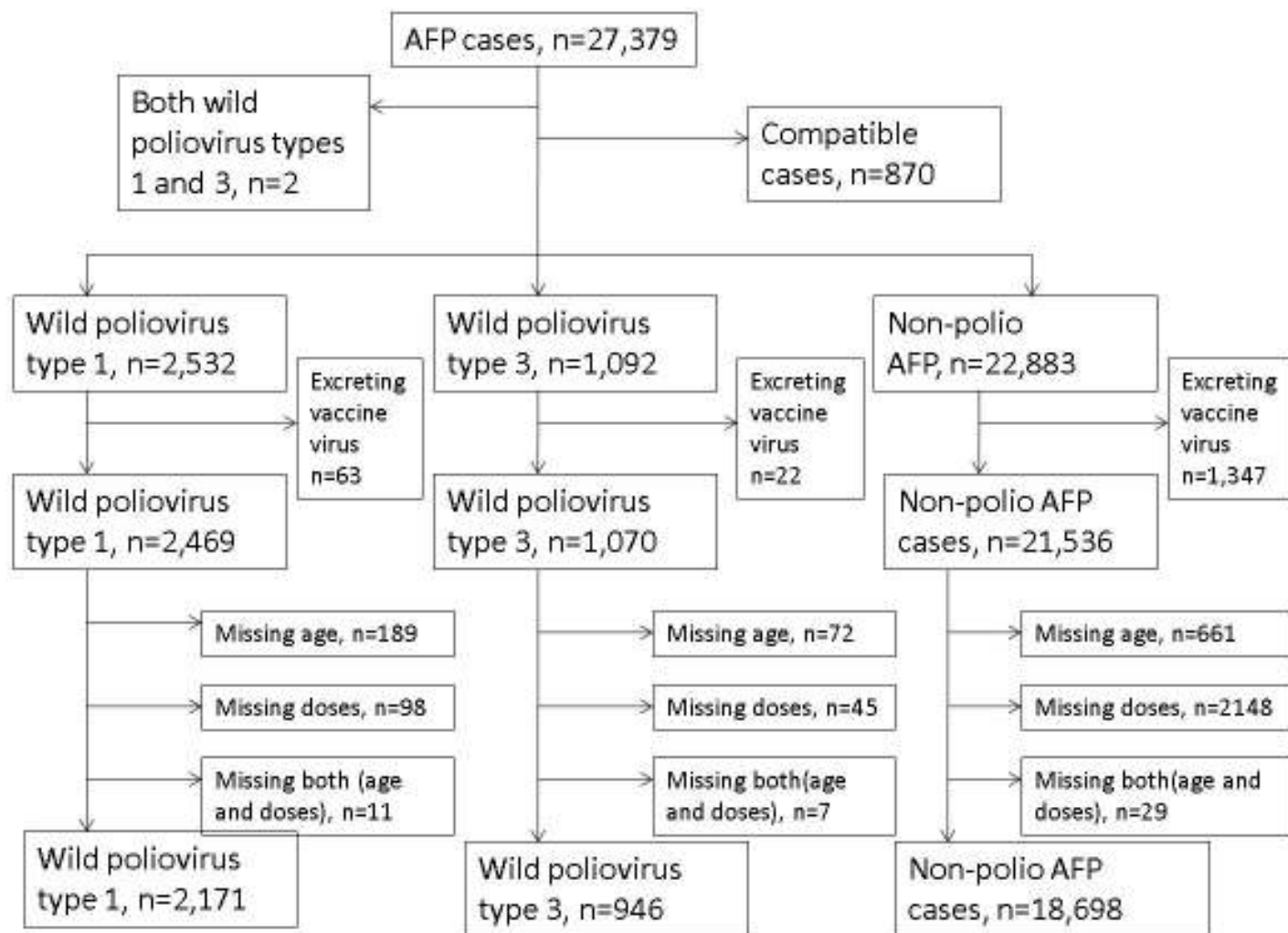


Figure 2.2 Flow chart illustrating the total number of AFP cases used in Chapter 5 (covers 1 January 2005 – 30 June 2009)

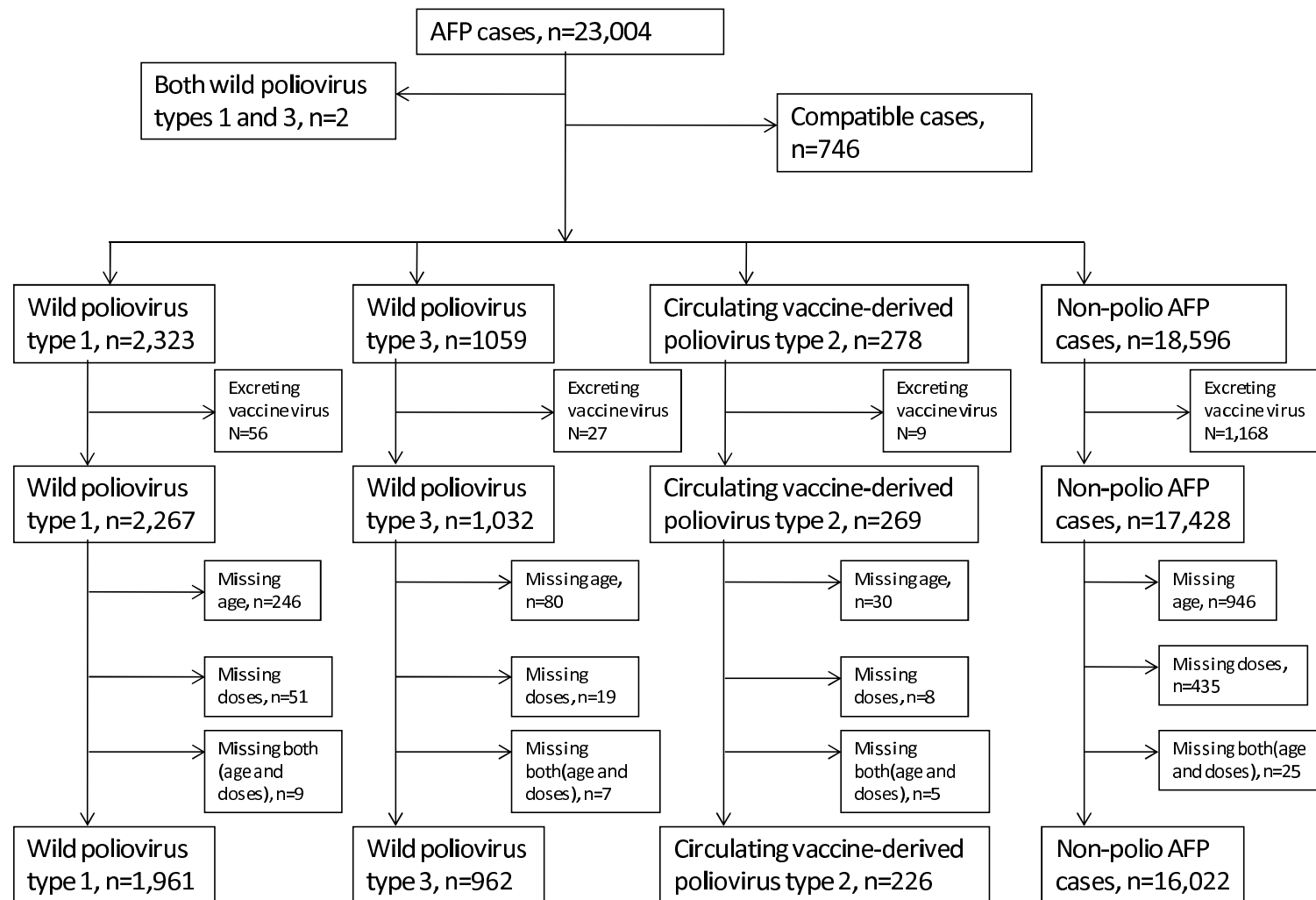
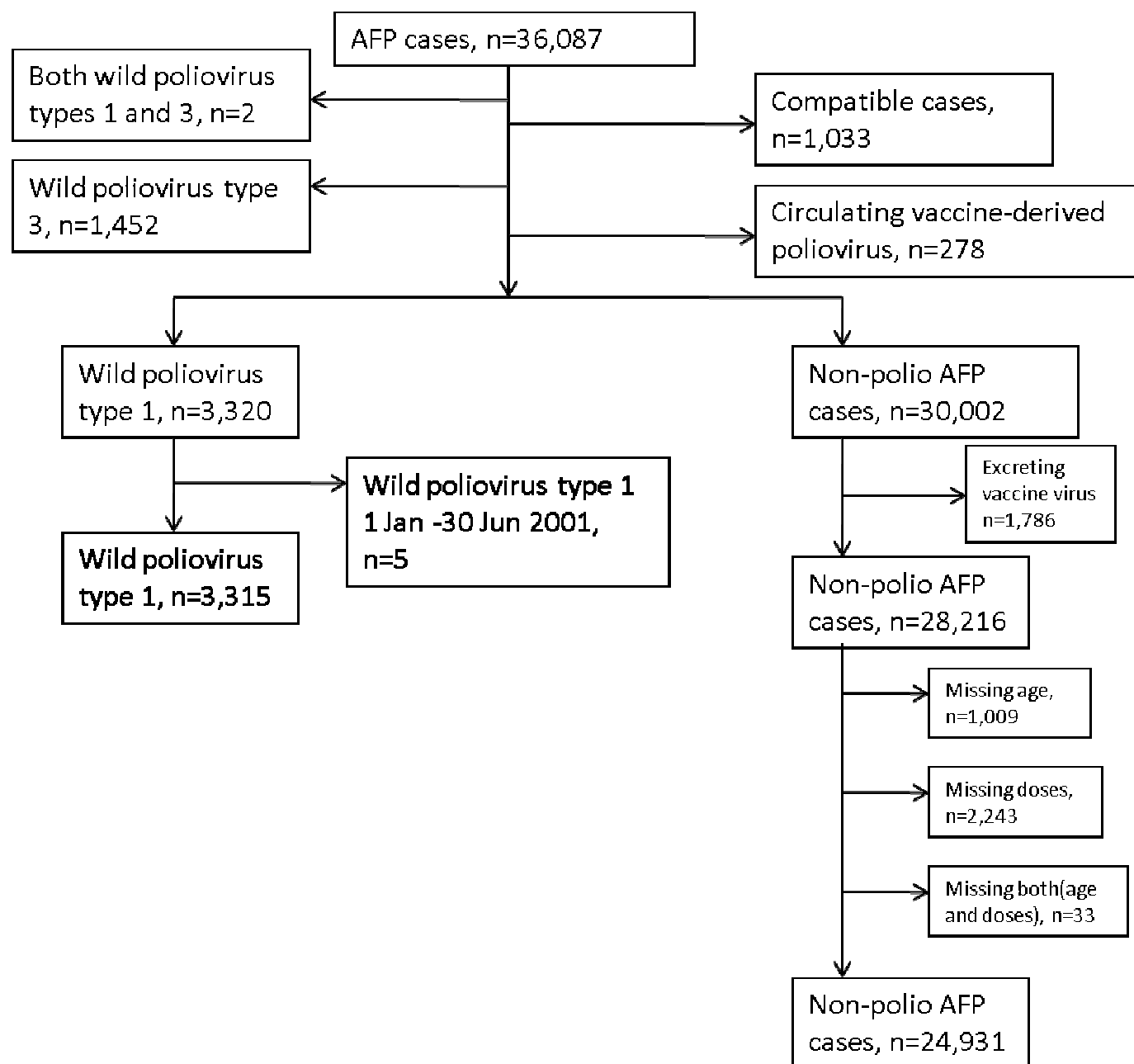


Figure 2.3 Flow chart illustrating the total number of AFP cases used in Chapter 6 (covers 1 January 2001 – 30 June 2009)



2.7 Descriptive analysis of the data

2.7.1 Frequency distribution of reported OPV doses

We examined the distribution of reported OPV doses by age (yearly categories), sex and geographical zone (see Tables 2.2 and 2.3 and Figure 2.4) and tested for any differences in the distribution by age, sex or zone using a Chi-Squared test. All AFP cases with a reported number of OPV doses were included.

Table 2.2 Number and distribution of reported OPV doses by age

OPV doses	Age (years), N (proportion)						Total
	0-1	1-2	2-3	3-4	4-5	>5	
0	465 (0.14)	1566 (0.17)	1238 (0.16)	575 (0.13)	240 (0.10)	465 (0.10)	4549 (0.14)
1	514 (0.15)	1056 (0.11)	818 (0.11)	442 (0.10)	183 (0.08)	257 (0.06)	3270 (0.10)
2	612 (0.18)	1302 (0.14)	1135 (0.15)	620 (0.14)	298 (0.12)	487 (0.10)	4454 (0.14)
3	745 (0.22)	1831 (0.19)	1447 (0.19)	835 (0.19)	424 (0.18)	943 (0.20)	6225 (0.19)
4	512 (0.15)	1466 (0.15)	1174 (0.15)	756 (0.17)	461 (0.19)	947 (0.20)	5316 (0.17)
5	301 (0.09)	1050 (0.11)	836 (0.11)	525 (0.12)	334 (0.14)	576 (0.12)	3622 (0.11)
6	140 (0.04)	489 (0.05)	413 (0.05)	266 (0.06)	171 (0.07)	375 (0.08)	1854 (0.06)
7	64 (0.02)	288 (0.03)	184 (0.02)	127 (0.03)	82 (0.03)	179 (0.04)	924 (0.03)
8	48	210	205	127	99	142	831

	(0.01)	(0.02)	(0.03)	(0.03)	(0.04)	(0.03)	(0.03)
9	11	89	92	65	25	66	348
	(<0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
10	9	74	94	74	26	106	383
	(<0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.02)	(0.01)
11	7	23	34	14	12	27	117
	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.01)	(<0.01)
12	2	11	27	21	18	40	119
	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.01)	(0.01)	(<0.01)
13	0	3	11	15	7	15	51
	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
14	0	1	10	8	6	9	34
	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
15	0	1	4	6	9	13	33
	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
16	0	0	4	8	7	2	21
	(0)	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
17	0	0	1	5	5	5	16
	(0)	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
18	0	0	1	2	1	4	8
	(0)	(0)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
19	0	0	0	0	1	2	3
	(0)	(0)	(0)	(0)	(<0.01)	(<0.01)	(<0.01)
20	0	0	0	0	0	3	3
	(0)	(0)	(0)	(0)	(0)	(<0.01)	(<0.01)
21	0	0	0	0	0	1	1
	(0)	(0)	(0)	(0)	(0)	(<0.01)	(<0.01)
22	0	0	0	0	0	2	2
	(0)	(0)	(0)	(0)	(0)	(<0.01)	(<0.01)
Total	3430	9460	7728	4491	2409	4666	32184

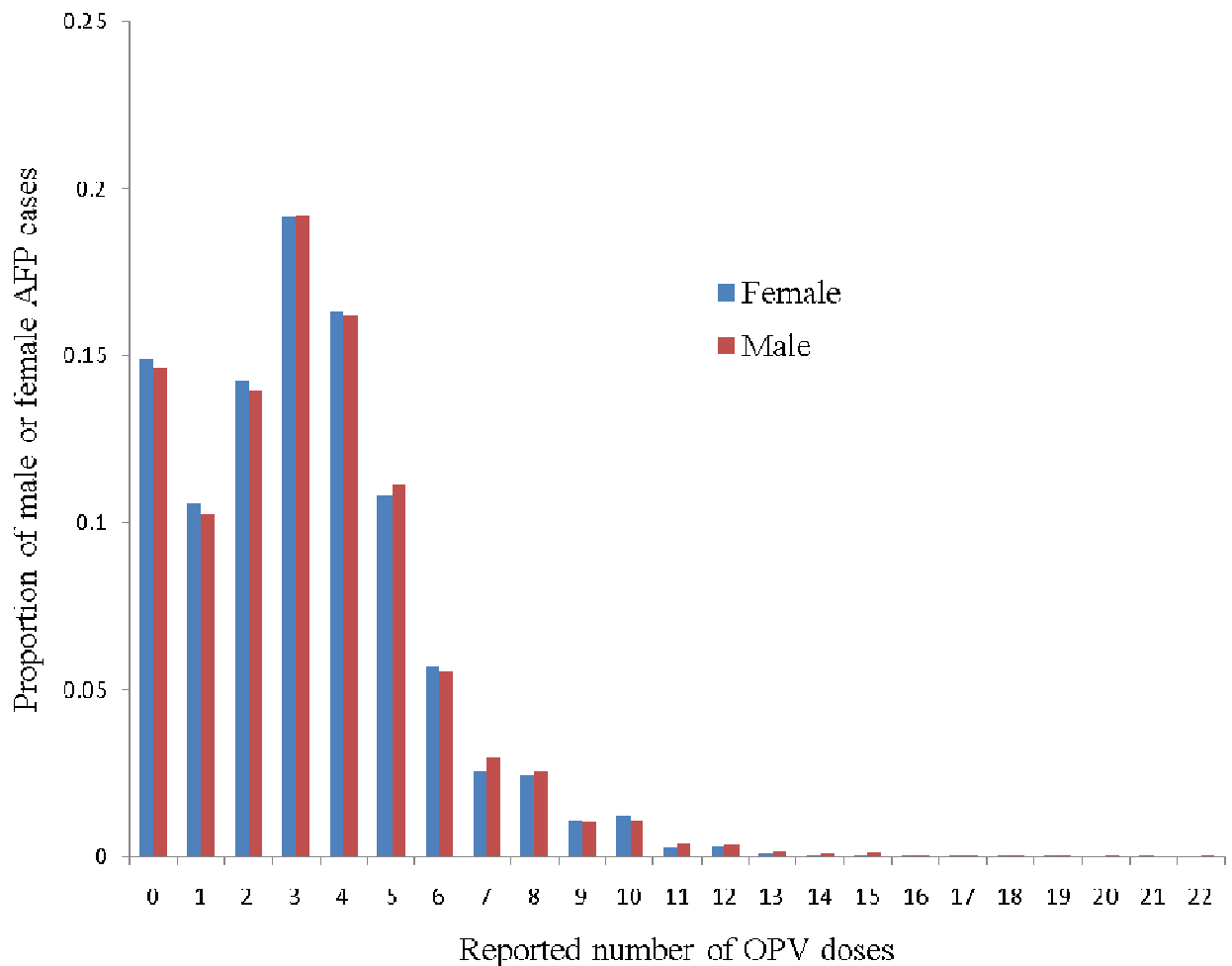
There was a wide range of reported doses from 0 to 22. It is notable that some children as young as 1 year old were reported to have had as many as 12 doses. This is theoretically possible if they have had all 4 available routine doses of OPV as there are some areas of the country that have had as many as 8 OPV campaigns within one year. However, it does seem unlikely that infants of this age would have received this many doses and it is possible that some parents/carers report that their children have received 'all that were available' and hence such a high number is recorded. It should also be noted that the absolute number of children reporting 10 doses was greater than those reporting 9 (383 vs. 348). This may indicate digit bias in the data, i.e. people are more likely to round the number of doses to a round number such as 10 if they are unsure of exactly how many doses were received.

There was a statistically significant difference between the distribution of OPV doses by age ($p < 0.001$). As would be expected, number of reported doses generally increased with age as children were exposed to more SIAs. However, AFP cases in children aged 1-3 were more likely to have received zero doses than those in other age groups. This result may have occurred due to confounding: the majority of polio cases are in this age group (Figure 2.5) and they are also more likely to have received zero doses (hence why they became infected with poliovirus). It is interesting that there was not a greater variation by age in the proportion of children that have received x number of doses. A much more detailed breakdown of these data might help to shed light on this as they are several potentially confounding factors

There was a statistically significant difference between the dose distributions in males and females ($p = 0.047$). Males were more likely to have received more OPV doses although the actual differences were negligible (Figure 2.4) and the statistically significant result was, in part, due to

high numbers (33685 AFP cases) and therefore there was considerable statistical power to find a difference.

Figure 2.4 Distribution of reported OPV doses split by gender



There was a statistically significant difference in the OPV dose distributions in different zones ($p < 0.001$) (Table 2.3). This would be expected since uptake of OPV has been considerably lower in Northern states than in the South of the country, as discussed in the Introduction.

Table 2.3 Distribution of reported OPV doses by geographical zone

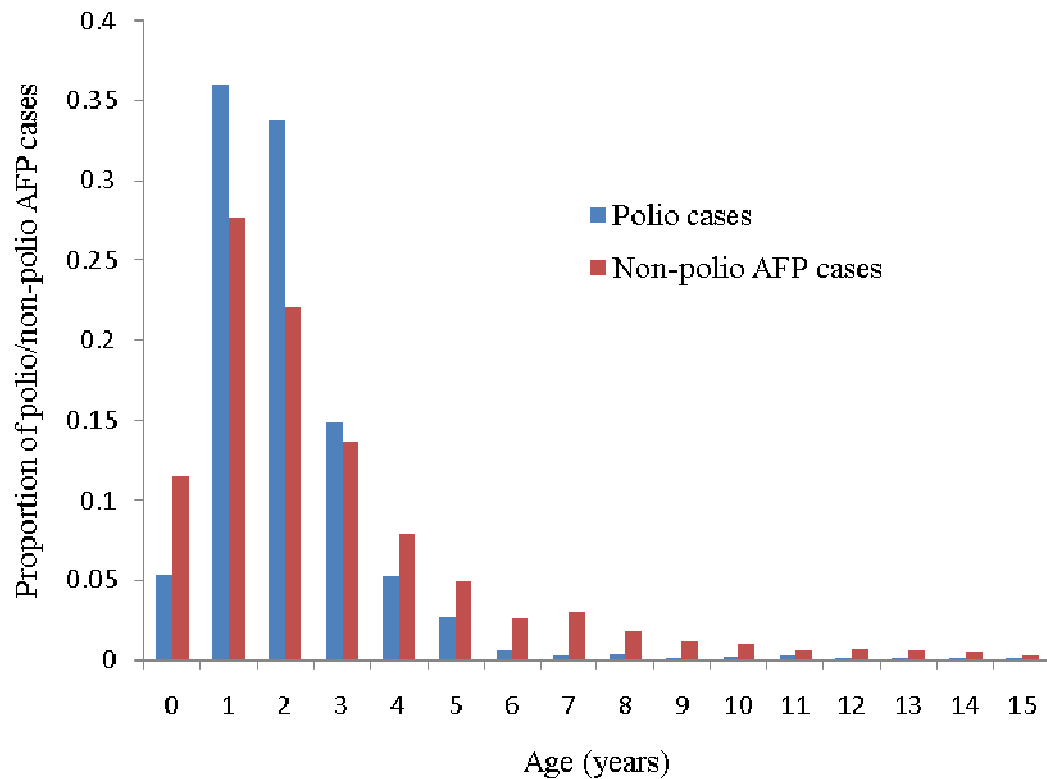
OPV doses	Zone, N (proportion)						Total
	North Central	North East	North West	South East	South South	South West	
0	267 (0.04)	744 (0.15)	3676 (0.29)	115 (0.05)	78 (0.03)	98 (0.02)	4978 (0.15)
1	351 (0.06)	660 (0.13)	1957 (0.16)	158 (0.07)	136 (0.04)	241 (0.05)	3503 (0.10)
2	569 (0.10)	892 (0.18)	2380 (0.19)	257 (0.11)	286 (0.09)	361 (0.08)	4745 (0.14)
3	1128 (0.19)	1024 (0.21)	2145 (0.17)	650 (0.27)	709 (0.23)	808 (0.17)	6464 (0.19)
4	1318 (0.22)	674 (0.14)	1273 (0.10)	587 (0.24)	689 (0.22)	936 (0.20)	5477 (0.16)
5	927 (0.16)	435 (0.09)	607 (0.05)	324 (0.13)	571 (0.18)	844 (0.18)	3708 (0.11)
6	548 (0.09)	184 (0.04)	268 (0.02)	165 (0.07)	314 (0.10)	416 (0.09)	1895 (0.06)
7	281 (0.05)	94 (0.02)	94 (0.01)	67 (0.03)	130 (0.04)	274 (0.06)	940 (0.03)
8	265 (0.04)	74 (0.02)	65 (0.01)	51 (0.02)	104 (0.03)	288 (0.06)	847 (0.03)
9	106 (0.02)	28 (0.01)	26 (<0.01)	20 (0.01)	35 (0.01)	138 (0.03)	353 (0.01)
10	127 (0.02)	48 (0.01)	43 (<0.01)	12 (<0.01)	26 (0.01)	133 (0.03)	389 (0.01)
11	18 (<0.01)	11 (<0.01)	5 (<0.01)	5 (<0.01)	7 (<0.01)	73 (0.02)	119 (<0.01)
12	29 (<0.01)	14 (<0.01)	4 (<0.01)	2 (<0.01)	17 (0.01)	54 (0.01)	120 (<0.01)
13	17 (<0.01)	2 (<0.01)	2 (<0.01)	0 (0)	1 (<0.01)	30 (0.01)	52 (<0.01)

14	5 (<0.01)	2 (<0.01)	0 (0)	2 (<0.01)	1 (<0.01)	24 (0.01)	34 (<0.01)
15	12 (<0.01)	2 (<0.01)	1 (<0.01)	0 (0)	2 (<0.01)	16 (<0.01)	33 (<0.01)
16	4 (<0.01)	0 (0)	2 (<0.01)	0 (0)	1 (<0.01)	14 (<0.01)	21 (<0.01)
17	3 (<0.01)	2 (<0.01)	0 (0)	0 (0)	0 (0)	11 (<0.01)	16 (<0.01)
18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (<0.01)	8 (<0.01)
19	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (<0.01)	3 (<0.01)
20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (<0.01)	3 (<0.01)
21	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<0.01)	1 (<0.01)
22	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (<0.01)	2 (<0.01)
Total	5975	4890	12548	2415	3107	4776	33711

2.7.2 Age distributions of AFP cases

Figure 2.5 shows the age distribution of AFP cases. Since surveillance for AFP cases focuses on children aged under 15 years, there may be a reporting bias and it is possible that there are many cases of AFP in adults which do not get reported. However, if we focus on children aged under 15 years, we can see that the majority of children reporting AFP are aged under 5 years. In particular, the vast majority of polio AFP cases ($>95\%$) are aged under 5 years. The age distribution of non-polio AFP cases is slightly more evenly spread and indeed the two distributions are statistically significantly different ($p<0.001$). However, the majority of non-polio AFP cases ($>82\%$) are also aged under 5 years.

Figure 2.5 Age distribution of polio and non-polio AFP cases



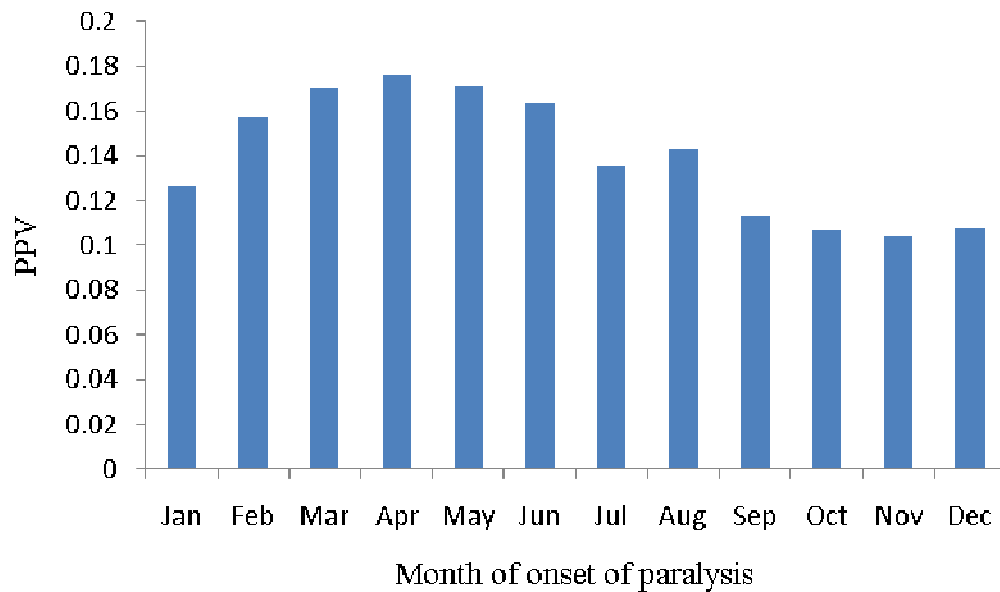
2.7.3 Positive Predictive Value (PPV)

Here we define the Positive Predictive Value (PPV) as the proportion of AFP cases that are polio cases. As the denominator we use the sum of the confirmed polio cases and those confirmed to be non-polio AFP while the numerator is the number of confirmed polio cases. PPV can be useful to indicate which AFP cases are more likely to be polio cases.

To examine the PPV by season, we looked at PPV by month of onset of paralysis (Figure 2.6).

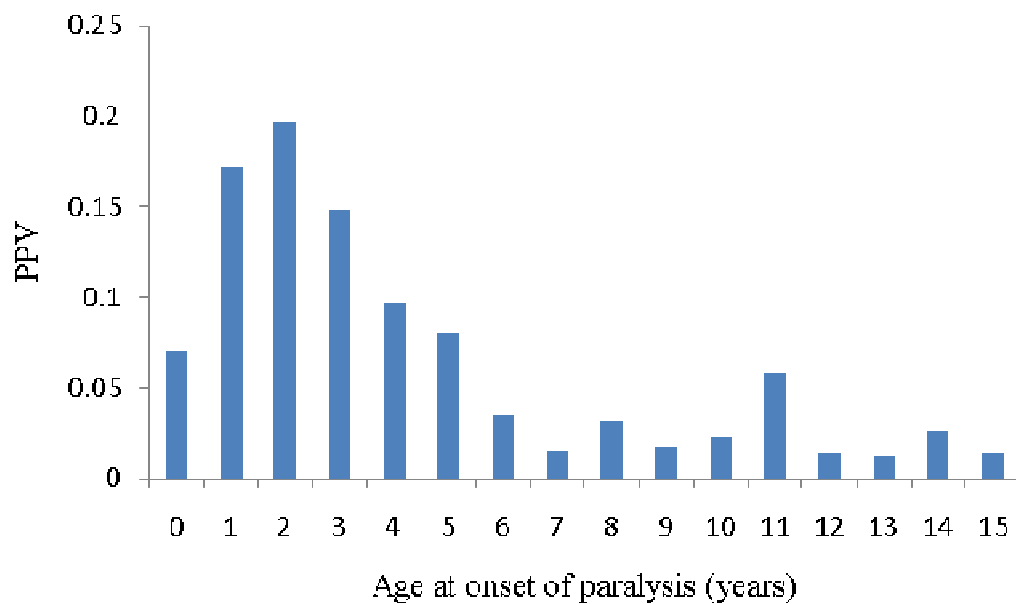
Polio cases (as a proportion of AFP cases) in Nigeria appear more likely to occur between February and August, on average, than at other times of the year.

Figure 2.6 Positive Predictive Value (PPV) by month of onset of paralysis



We also examined PPV by age (yearly age categories) (Figure 2.7)

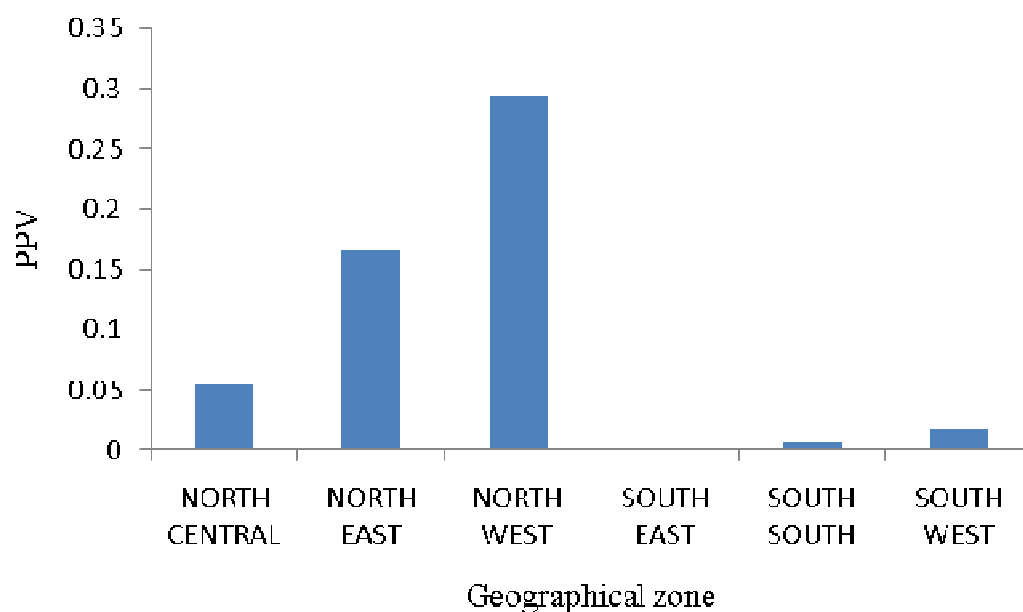
Figure 2.7 Positive Predictive Value (PPV) by age at onset of paralysis



AFP in a child aged 5 years and under is more likely to be a polio case than those in older children.

We also examined PPV by geographical zone (Figure 2.8).

Figure 2.8 Positive Predictive Value (PPV) by geographical zone



Of those that were confirmed to be either polio or non-polio AFP, in the North West zone nearly a third were polio cases. In contrast, less than 1% in the South East and South South zones were polio cases. Clearly, AFP cases in the Northern zones are more likely to be polio cases than those in the South.

The North West zone in particular is very different from the rest of the country as can be seen in Figure 2.8 and Table 2.3. It has experienced by far the largest burden of polio in recent years and large proportions of the population have received zero or very few doses of OPV. As described

in the Introduction, it was in this zone that rumours regarding the safety of OPV were started leading to a drop in vaccine uptake. There are also clear links between the rumours and the predominant religion in this zone (Islam). Other than Kano city, population density in the North West zone is low. There may be other differences between this zone and others in Nigeria which cannot be gleaned from our data and a more detailed investigation into the healthcare systems and their ability to reach all children in this zone might shed further light on this.

2.7.4 Differences by gender

We examined the distribution of polio and non-polio AFP cases by gender and by gender in different age groups and geographical zones. We used a Fisher's exact test to test the null hypotheses of (a) the ratio of males to females (i.e. number of males/number of females) was equal to 1 and (b) the same ratio amongst polio AFP cases was equal to that amongst non-polio AFP cases.

There were more male than female polio cases (57% versus 43%, $p\text{-value} < 0.001$ for a ratio different from 1) (Table 2.4). The proportion of male and female non-polio AFP cases was very similar and there was no difference between the ratio of males to females in polio and non-polio AFP cases ($p=0.37$) (Table 2.4).

Table 2.4 Number and proportion of polio and non-polio AFP cases that are male and female

AFP type	Male (proportion)	Female (proportion)	Ratio (male/female)	P-value testing if ratio =1
Polio	2863 (0.566)	2196 (0.434)	1.30	<0.001
Non-polio	17280 (0.573)	12890 (0.427)	1.34	<0.001
P-value for difference in ratio between polio and non-polio AFP cases				0.37

Examining the gender split by age group, there were more males with both polio and non-polio AFP in each age group. Amongst polio cases, the ratio was not always significantly different from 1 although this may have been due to small numbers in some age groups and therefore a lack of statistical power. There were no significant differences between the ratio in polio and non-polio AFP cases in any age groups other than the 3-4 years group ($p=0.037$) where the ratio of males to females was higher in non-polio AFPs than polio AFPs (ratio=1.40 vs. 1.18) (Table 2.5).

Table2.5 Number and proportion of polio and non-polio AFP cases that are male and female by age group

AFP type	Age (years)	Male (proportion)	Female (proportion)	Ratio	P-value testing if ratio =1	P-value testing if ratio for age group among polio cases = that among non- polio cases
Polio	0-1	128 (0.51)	123 (0.49)	1.04	0.75	0.51
	1-2	931 (0.56)	737 (0.44)	1.26	<0.001	0.79
	2-3	925 (0.59)	642 (0.41)	1.44	<0.001	0.65
	3-4	374 (0.54)	317 (0.46)	1.18	0.03	0.037
	4-5	133 (0.54)	114 (0.46)	1.17	0.23	0.076
	>5	123 (0.57)	93 (0.43)	1.32	0.04	0.48
Non- polio	0-1	1779 (0.53)	1559 (0.47)	1.14	<0.001	
	1-2	4452 (0.55)	3580 (0.45)	1.24	<0.001	
	2-3	3737 (0.58)	2665 (0.42)	1.40	<0.001	
	3-4	2324 (0.58)	1657 (0.42)	1.40	<0.001	
	4-5	1367 (0.60)	918 (0.40)	1.49	<0.001	
	>5	2994 (0.60)	2036 (0.40)	1.47	<0.001	

Within each geographical zone, more males than females were polio and non-polio AFP cases (Table 2.6). The ratio of males to females was significantly different from 1 in all zones for non-polio AFP cases and in all northern zones for polio cases. In southern zones, the ratio was greater

than 1 but not significantly so for polio cases. It is likely that this was due to small numbers and therefore a lack of statistical power to find a difference. There were no significant differences in any of the zones between the ratios for polio and non-polio AFP cases. Sometimes a gender bias is associated with different cultures or religions. Interestingly, there was no obvious difference between the Northern and Southern zones in terms of the bias despite the people following different religions in these two areas. A further analysis which can separate different types of populations within Nigeria more specifically would be interesting as would an examination of any gender biases in AFP datasets from other countries.

Table2.6 Number and proportion of polio and non-polio AFP cases that are male and female by geographical zone

AFP type	Zone*	Male (proportion)	Female (proportion)	Ratio	P-value testing if ratio =1	P-value testing if ratio for zone among polio cases = that among non- polio cases
Polio	NC	189 (0.56)	148 (0.44)	1.28	0.026	0.61
	NE	488 (0.57)	374 (0.43)	1.30	<0.001	0.82
	NW	2118 (0.57)	1618 (0.43)	1.31	<0.001	0.91
	SE	4 (0.57)	3 (0.43)	1.33	0.71	>0.99
	SS	15 (0.68)	7 (0.32)	2.14	0.089	0.40
	SW	49 (0.52)	46 (0.48)	1.07	0.76	0.17
Non-polio	NC	3359 (0.58)	2479 (0.42)	1.35	<0.001	
	NE	2447 (0.56)	1912 (0.44)	1.28	<0.001	
	NW	5084 (0.57)	3904 (0.43)	1.30	<0.001	
	SE	1479 (0.56)	1162 (0.44)	1.27	<0.001	
	SS	1919 (0.59)	1351 (0.41)	1.42	<0.001	
	SW	2992 (0.59)	2082 (0.41)	1.44	<0.001	

*NC=North Central, NE=North East, NW=North West, SE=South East, SS=South South, SW=South West

Our results indicate that there might be a bias in reporting of AFP. Since the ratio of males to females reporting AFP is consistent across polio and non-polio AFP cases, there is a possibility that parents/carers are more likely to report boys with AFP than girls. This type of sampling bias

can sometimes be associated with religion. Although the population in the north of Nigeria is largely Muslim and in the South, largely Christian, the ratio of males to females is consistent across zones suggesting that this is a countrywide effect and not strongly associated with one religion over another.

3. Statistical Methods

3.1 Estimation of vaccine efficacy

Matched case control studies were used to estimate the per dose efficacy of the different OPVs against paralysis by each poliovirus serotype. Polio cases were 1:1 matched with controls randomly selected from the database of children with non-polio AFP based on the age at onset of paralysis (within 6 months), the date of onset of paralysis (within 6 months) and location. When matching by location, the same LGA was used for the analysis in Chapter 4 since several SIAs in 2007 had been carried out at the LGA level and results were found to be sensitive to this. In Chapter 5, matching by location was carried out at the state level since matching by LGA in the analysis of cVDPV2 cases excluded a large proportion (74%) of cases. In Chapter 5, cases were also matched by sex. Sensitivity of results to the matching criteria was tested for. These matching criteria were chosen to maximize the number of case-control pairs, whilst minimizing potential biases introduced by a failure to control for differences in exposure between cases and controls. Non-polio AFP cases were considered appropriate controls because AFP cases were investigated prior to confirmation of polio status ensuring blinding of both investigators and parents/carers during dose reporting interviews.

3.1.1 Conditional logistic regression of 1:1 matched cases and controls

Conditional logistic regression of the 1:1 matched data was used to relate the log-odds of disease to the number of doses of vaccine.

The probability that a child who has received m doses of mOPV against serotype i and t doses of tOPV is protected against paralysis by that serotype is:

$$p_i(m,t)=1- (1-v_i)^t(1-v_m)^m \quad (\text{eq. 1})$$

where v_m and v_t give the efficacy of mOPV and tOPV, respectively, against the serotype under consideration.

The efficacy of mOPV1, mOPV3 and tOPV was estimated using conditional logistic regression, which estimates the $\ln(\text{odds})$ of paralysis by serotype i as

$$\ln(\text{odds}) = \beta_1 m + \beta_2 t + E$$

where the β 's are the regression coefficients and each matched case-control pair has a particular level of exposure to wild poliovirus, E , which is unknown and is eliminated from the likelihood by conditioning on the pair having one case^{7, 75}. Vaccine efficacy is related to the relevant regression coefficient by $1 - e^\beta$.

In Chapter 5, parallel analyses were carried out in which vaccine doses of all three vaccine types were entered into each model (i.e. each of the three models, one for each serotype). This was to check for any positive or negative effects of the vaccines that are not used to target that specific serotype. Assuming no negative or positive effects, it was preferable to exclude these from the main analyses since collinearity in the data would increase estimated parameter standard errors and reduce the accuracy of our estimated efficacies of main interest.

To assess the validity of reporting a constant efficacy per dose received of tOPV, the estimated number of tOPV doses received was entered as a continuous and categorical variable in separate models and these models were compared via a likelihood ratio test. The same test was carried out to assess a constant efficacy per dose of mOPV1 and mOPV3 against serotype 1 and 3 respectively. Because of the method of estimating the number of doses of mOPV1, mOPV3 and tOPV received by AFP cases, fractional dose numbers were possible. Dose number was therefore rounded to the nearest whole dose in the categorical analysis of mOPV1 (Chapter 5) and tOPV efficacy against serotypes 1 and 3, and to the nearest half-dose for mOPV1 (Chapter 4) and tOPV against serotype 2 and mOPV3 (due to the smaller number of doses of these vaccines distributed).

Under the assumption of a constant efficacy per dose of vaccine received, the probability of protection following multiple doses is estimated using (eq. 1). For example, if a child has received 2 doses of mOPV1 and 3 of tOPV then, using the efficacy estimates presented in Chapter 4:

$$\text{Probability of protection} = p = 1 - (1 - 0.67)^2 (1 - 0.16)^3 = 0.94$$

Variation in vaccine efficacy by zone, year and age was assessed by including appropriate interaction terms in the regression model.

3.1.2 Sensitivity of estimated vaccine efficacy to routine coverage assumptions

When estimating vaccine efficacy above, we used the reported number of doses received and attributed those doses to tOPV, mOPV1 and mOPV3 as described above making the assumption that all doses were received through SIAs and none through routine immunization. This is a very strong assumption which is wrong to varying degrees in different parts of the country. Since data on the number of routine OPV doses received was not available for individuals, we have tested the sensitivity of the vaccine efficacy estimates to this assumption using data from the NICS⁶⁰ which reports the proportion of children by state and zone that have received routine doses of OPV.

Routine immunisation in Nigeria consists of 4 doses of tOPV administered at birth, 6, 10 and 14 weeks of age. The percentage of children receiving 3 routine doses by 52 weeks of age by state, as reported in the NICS⁶⁰, was used (since coverage with the birth dose is typically low, the NICS reports the percentages of children receiving 3 doses instead of 4). In particular, the NICS reports the following proportions of children receiving 3 routine doses by 52 weeks of age by zone: North West : 21.4%, North East : 28.3%, North Central: 36.0%, South West: 53.7%, South South: 41.8%, South East: 47.1%⁶⁰. To test the sensitivity of our vaccine efficacy estimates to the assumption that all vaccine doses in our analysis were received from SIAs we firstly assumed that the 3 routine doses of tOPV had been received by a

proportion of the population in each zone according to the NICS data. The specific individuals that would be in this proportion were chosen randomly. The remaining doses for each of those individuals (i.e. their total doses minus the 3 doses that have just been attributed to tOPV as routine doses) were then assumed to have been received through SIAs and the number of mOPV1, mOPV3 and tOPV doses estimated as described above. We ignored routine coverage with fewer than 3 doses of tOPV, assuming that children were more likely to receive either all 3 doses or none at all. Vaccine efficacy was calculated using conditional logistic regression as described above. This analysis was repeated 100 times (with the individuals that made up the proportion that received routine doses being randomly assigned each time) and the percentiles for the estimated efficacy were recorded. This analysis was carried out for each of the three analyses of efficacy (i.e. one for each serotype and the vaccines that protect against paralysis due to that serotype). Each time a completely random set of children were selected to have received routine doses regardless of whether they were cases or controls.

The analysis was then repeated for the estimates of the efficacy of tOPV against cVDPV2 assuming only control children received routine tOPV doses, on the basis that cVDPV2 cases may not have received this effective vaccine (instead, they are more likely to have received the monovalent vaccines against serotypes 1 and 3). Similarly, analyses were repeated for WPV1 and WPV3 assuming that only cases had received the routine tOPV doses on the basis that they were less likely to have received the more effective mOPVs against type 1 or 3. These analyses attributing routine doses to either all cases or all controls make extreme assumptions and the reality is likely to be between these and the scenario in which routine doses are assumed to be split equally between cases and controls.

3.2 Estimation and analysis of vaccine coverage (Chapter 4)

Vaccine coverage was estimated from the dose-reporting of non-polio AFP cases. Our aim was to test if vaccine coverage in different zones in Nigeria had changed between 2005 and 2007. All non-polio AFP

cases aged under 5 years reported in 2005 were categorised by the number of doses of OPV received (as reported by parents/carers): 0, 1-3 or >3 doses, state of residence and single years of age. This categorisation was carried out by total doses received to obtain estimates of coverage regardless of vaccine type. This resulted in 185 sets of proportions in each dose category ($185 = 37 \text{ states} \times 5 \text{ age categories}$), represented by 185 vectors each with three entries (one for each dose category as defined above). The proportion of children in each of the three dose categories was estimated for each of the 6 geographic zones in Nigeria as a weighted sum over age categories and states to reflect their underlying age distribution and population size^{76, 77}. For example, let w_1 be the fraction of the under 5 year old population that are in the first age category (0-1 years old), w_2 be the fraction in the second age category (1-2 years old) etc up to w_5 (as taken from the Demographic Health Survey which lists the proportion of the population in each age category⁷⁶). Then let the vector of proportions in each dose category for the

first age category in one state equal $\begin{pmatrix} p_{11} \\ p_{12} \\ p_{13} \end{pmatrix}$ where p_{11} is the proportion of children aged 0-1 years old

reporting 0 doses, p_{12} is the proportion of children aged 0-1 years old reporting 1-3 doses and p_{13} is the proportion of children aged 0-1 years old reporting more than 3 doses. Similarly, the vector for children in

the second age category (1-2 years old) is $\begin{pmatrix} p_{21} \\ p_{22} \\ p_{23} \end{pmatrix}$ and so on for all five age categories. We then estimate

the weighted proportions of children in each dose category for that state to be

$w_1 \begin{pmatrix} p_{11} \\ p_{12} \\ p_{13} \end{pmatrix} + w_2 \begin{pmatrix} p_{21} \\ p_{22} \\ p_{23} \end{pmatrix} + \dots + w_5 \begin{pmatrix} p_{51} \\ p_{52} \\ p_{53} \end{pmatrix}$. The resulting vector was estimated for each state (i.e. 37 vectors in

total). Vectors for each of the 6 zones in Nigeria were then estimated similarly using fractions of the population in each zone which are in each state within that zone (as taken from the census⁷⁷). For details

of which states fall into which of the 6 zones, see Appendix Table A1a. This process was repeated for non-polio AFP cases reported in 2007.

In order to calculate the variance of these weighted estimates, a variance-covariance matrix was calculated for each of the 185 vectors of proportions assuming that the proportions follow a multinomial distribution. From these, a weighted variance-covariance matrix was calculated for each of the 6 zones (again using age and population size weights as described above).

The differences in proportions between 2005 and 2007 in each zone were calculated by subtracting the 2005 vector of proportions from that for 2007 (for each zone in turn). The variance-covariance matrices for these difference vectors were calculated by adding the variance-covariance matrix for 2005 to that for 2007. This was under the assumption that the data from the two years were independent and therefore $\text{Var}(X-Y)=\text{Var}(X)+\text{Var}(Y)$. This assumption is certainly violated to some degree although assuming that the covariance is positive, it will over estimate the variance (since without the assumption of independence $\text{Var}(X-Y)=\text{Var}(X)+\text{Var}(Y)-\text{cov}(X,Y)$) and therefore our test will be more conservative and less likely to find differences that exist.

If there is no difference between two proportions X and Y , then the test statistic $T = (X-Y)^2/(\text{Var}(X-Y))$ is asymptotically χ^2 distributed with 1 degree of freedom (under the assumption that the central limit theorem holds and expected values are all greater than 5). In this case we use the multidimensional equivalent $T = \underline{\mathbf{d}}^T \underline{\mathbf{V}}^{-1} \underline{\mathbf{d}}$, where $\underline{\mathbf{d}}$ is the 3x1 vector of the differences in weighted proportions between 2005 and 2007 and $\underline{\mathbf{V}}$ is the 3x3 weighted variance-covariance matrix of the differences. In this case the test statistic is asymptotically χ^2 distributed with 2 degrees of freedom (under the assumption that the central limit theorem holds and expected values are all greater than 5).

3.3 Estimation of vaccine-induced population immunity

Vaccine-induced population immunity and the confidence intervals around these results were estimated using slightly different methods in Chapters 4 and 5. In Chapter 4, immunity levels were only estimated against paralysis by one serotype: wild poliovirus serotype 1. Since the efficacy of tOPV against this serotype has been found to be low (see Chapter 4 results), routine immunization with tOPV would have little impact on the immunity levels and thus it was possible to estimate immunity levels based solely on dose numbers that assumed that all doses had been received through SIAs (where many of the doses administered were of mOPV1 with its relatively high efficacy). However, when estimating immunity levels against paralysis by cVDPV2, these were very sensitive to routine immunization for two reasons: the relatively high efficacy of tOPV against paralysis by this serotype (see Chapter 5 results) and the fact that tOPV is the only vaccine administered during the study period that induces immunity against cVDPV2 and as it has been used infrequently in SIAs, doses received during routine immunization will have had a greater relative impact. Therefore estimates of population immunity in Chapter 5 were calculated accounting for routine immunization rates by state and the different methods are described separately below.

3.3.1 Estimation of vaccine-induced population immunity in Chapter 4

Population immunity induced through direct immunization with OPV against paralysis among children under 5 years old was estimated by state and year based on the reports of the number of OPV doses received by non-polio AFP cases and the estimated efficacy of the vaccines^{48, 75}.

The fraction of children protected against paralysis by serotype i poliovirus is given by

$$F_i = \frac{\sum_j n_j p_i(m_j, t_j)}{\sum_j n_j} \quad (\text{eq. 2})$$

where j is an index over all unique possible combinations of doses received, n_j is the number of children that have received the j^{th} combination of doses, t_j is the estimated number of tOPV doses in the j^{th} combination, m_j is the estimated number of mOPV doses of the relevant serotype in the j^{th} combination and $p_i(m,t)$ is defined in equation 1.

Estimates were weighted by single years of age to reflect the underlying age distribution of the population⁷⁶ and estimates by zone weighted to reflect the underlying population distribution by state⁷⁷. This quantity, F , is calculated for each of the five age groups within a state and an average of these five numbers is calculated weighting each number by the proportion of children in Nigeria under five that are in that age group, i.e.

$$\text{Fraction protected} = w_1F_1 + w_2F_2 + w_3F_3 + w_4F_4 + w_5F_5$$

Where F_l is the fraction protected in the age group 0-1 years and w_l is the fraction of the under-five population that are 0-1 years old as before⁷⁶. Estimates by zone were based on a weighted average using weights derived from the proportion of the population of the whole zone that are in each state⁷⁷.

3.3.2 Estimation of confidence intervals around vaccine-induced population immunity estimates in Chapter 4

Vaccine-induced immunity is a function of both reported coverage and estimated efficacy of mOPVs and tOPV (eq. 2). Confidence intervals around the estimates of vaccine-induced immunity were derived using the Delta method. Assuming that the errors around each parameter in F are asymptotically normally distributed with mean 0 and known variance and that the Taylor expansion of F is dominated by the first-order terms, the variance around F is given by:

$$\text{Var}(F) = (\nabla F(\alpha, \beta, m_0, \dots, m_D, t_0, \dots, t_D, n_0, \dots, n_D))^T \Sigma (\nabla F(\alpha, \beta, m_0, \dots, m_D, t_0, \dots, t_D, n_0, \dots, n_D))$$

where Σ is the variance-covariance matrix of the parameters, D is the total number of doses reported to have been received and ∇F are the partial derivatives of F with respect to each parameter. $\text{Var}(\alpha)$, $\text{Var}(\beta)$ and $\text{Cov}(\alpha, \beta)$ are all given directly from the conditional logistic regression. $\text{Var}(m_i) = (m_i t_i) / (m_i + t_i)$, $\text{Var}(t_i) = (m_i t_i) / (m_i + t_i)$ and $\text{Cov}(m_i, t_i) = -(m_i t_i) / (m_i + t_i)$, these variances and covariances assume that the probability that each dose that a child received has a fixed, constant probability of being either mOPV1 or tOPV over time and these probabilities follow a binomial distribution. $\text{Var}(n_i) = N p_i (1 - p_i)$ where $p_i = n_i / N$ and $\text{Cov}(n_i, n_j) = -N p_i p_j$, these variances and covariances assume that the number of children that receive a particular combination of doses follows a multinomial distribution. We assume the covariances $\text{Cov}(\alpha, n_i)$, $\text{Cov}(\beta, n_i)$, $\text{Cov}(m_i, n_i)$ and $\text{Cov}(t_i, n_i)$ are zero for all i . The variance of F was calculated for each of the 5 age groups in each of the 37 states and overall variances for each zone were calculated and weighted to match the underlying age distribution and population size^{76, 77}.

3.3.3 Estimation of vaccine-induced population immunity and resulting confidence intervals in Chapter 5

In Chapter 5, population immunity was initially estimated as described above (3.3.1). The impact of routine immunization coverage with tOPV on population immunity to poliovirus was accounted for based on coverage reported by the NICS using a hierarchical model to assign tOPV doses to non-polio AFP cases.

To estimate the fraction of children protected against paralysis by serotype i poliovirus and attack rates per 100,000 ‘susceptible’ children, we used a hierarchical model implemented with the WinBUGS software⁷⁸. In each state, the number of doses of mOPV1, mOPV3 and tOPV vaccine received by non-polio AFP cases was modeled as a mixture distribution with two components, where the mixture probability r_s equals the fraction of children receiving 3 routine doses of tOPV in state s according to the NICS⁷⁹ (as described above). In the first component, which was associated with the probability $1 - r_s$, the number of doses was modeled as a constrained random vector $(m1, m3, t)$ for each control individual k .

Let nD be the total number of vaccine doses reported for k . The components of the vector $(m1, m3, t)$ are then defined as follows. Let $(e1, e3, eT)$ be the calculated rounds of exposure for the three vaccine types, respectively. Let $(q1, q3, qT)$ be the vector of corresponding probabilities obtained by dividing the elements of $(e1, e3, eT)$ with their sum. In the rare cases where the sum of $(e1, e3, eT)$ equals zero we set $qT = 1$ to reflect the fact that any reported doses must have been obtained through routine immunization activity involving only tOPV vaccine. The number of doses $m1$ is modeled as a truncated binomial $(nD, q1)$ variate by setting $m1$ equal to $e1$ for the binomial events where $m1 > e1$. The number of doses $m3$ is analogously defined by truncating a binomial $(nD, q3)$ variate. Finally, the number of doses t is defined as $nD - m1 - m3$. In the second component, which was associated with probability r_s , the number of doses was modeled as a sum of routinely administered tOPV vaccine rounds and a random vector $(m1, m3, t)$. The number of routinely administered tOPV doses equals 3 in those cases where the number of reported doses is at least 3, and equals the number of reported doses otherwise. The random vector of doses $(m1, m3, t)$ is defined analogously to the above description, except that the sum of its components is set equal to the total number of reported doses nD minus the routinely administered doses.

In addition to the uncertainty related to the dose distribution for each individual, it was necessary to represent the uncertainty about the estimated vaccine efficacy given the information provided by the conditional logistic regression. Each regression parameter was assigned a normal distribution with mean equal to the maximum likelihood estimate and standard deviation equal to the standard error of the estimate. Thus, this part of our hierarchical model represents the uncertainty remaining about the vaccine efficacy given the results of the conditional logistic regressions described above.

By fitting the hierarchical model to each annual dataset from each state, it was possible to simulate the marginal posterior distribution of any function of the vaccine efficacy and the doses administered. The

marginal posterior distribution efficiently takes into account the uncertainty related to all nuisance parameters and enables inferences to be made similarly to a profile likelihood method. The two functions of interest here were the proportion of the under-five population protected through direct immunisation (eq. 2) and the attack rates per 100,000 ‘susceptible’ children. By simulating 10,000 values from the marginal distribution we constructed the estimates using the means and the 95% Bayesian confidence intervals by the 2.5% and 97.5% percentiles of the simulated values. The convergence of the individual simulations was assessed using the standard tools available in WinBUGS and the simulated chains were concluded to have desirable characteristics.

3.3.4 Additional analyses using population immunity estimates

In Chapter 4, any increases found in population immunity levels between 2005 and 2007 could have been due to either a) an increase in vaccine coverage (more OPV doses being received and therefore immunity levels increasing) or b) a greater protective efficacy of mOPV1 introduced in 2006 or c) a combination of a) and b). To assess this, we recalculated the population immunity levels assuming that tOPV had been used in all rounds. This gave us an estimate of population immunity assuming that the efficacy of mOPV1 was equivalent to that of tOPV and hence what changes in immunity were due solely to changes in vaccine coverage. We then subtracted these recalculated immunity levels from those using our different estimates for the efficacies of tOPV and mOPV1 to estimate the contribution of mOPV1 to the changes in immunity levels. Thus we estimated the relative contribution of changes in vaccine coverage and increased efficacy of mOPV1 to the population immunity levels.

In Chapter 4, correlation between the proportion of children aged under 5 years in a state protected by vaccination and reported numbers of type 1 polio cases was estimated using Spearman’s rank correlation coefficient. In Chapter 5, changes in population immunity were assessed by fitting a linear trend to the yearly estimates, weighting each year by the sample size.

3.4 Sensitivity of testing for poliovirus among AFP cases (Chapter 4)

The sensitivity of testing for wild poliovirus among AFP cases with two stool samples was estimated for the dataset used in Chapter 4 using published methods⁸⁰. Sensitivity of testing for vaccine-derived poliovirus (Chapter 5) was not estimated since testing methods for vaccine-derived poliovirus changed in 2007 (halfway through the study period).

A method for evaluating the sensitivity of testing for poliovirus among AFP cases with two stool samples was developed and published by Gary et al. in 1997⁸⁰ and is used in this thesis. The method assumes that the laboratory test for poliovirus is 100% specific and that the sensitivities of the test for each stool are equal, which is a reasonable assumption provided that the time between collection of each sample is short (in AFP surveillance, the stool samples are typically collected between 24 and 48 hours apart and this was the case for 99% of AFP cases in our dataset). The maximum likelihood estimate of the sensitivity to detect poliovirus from a single stool is given by $S = 2N_{11}/y$ and the prevalence of infection is $y^2/(4N_{11}T)$, where $y = 2N_{11} + N_{10} + N_{01}$ = number of positive specimens, N_{10} = number of people whose first specimen was positive and the second was negative, N_{01} = number of people whose first specimen was negative and the second was positive, N_{11} = number of people with two positive specimens, and T = number of AFP cases. The person sensitivity was defined as the probability that at least one specimen from a child with polio will have a positive test result. As two specimens were collected, person sensitivity was the complement of the probability that both specimens were negative = $1 - (1 - S)^2$ (assuming that the two specimens were independent).

The probability of misclassifying a case from the AFP database who was paralysed by wild poliovirus as a child without polio was therefore given by prevalence * (1 – person sensitivity).

3.5 Clinical characteristics (Chapter 5)

Clinical characteristics of AFP associated with WPV1, WPV3 and cVDPV2 isolation were compared using Fisher's exact test (for categorical variables) or a t-test (for age, allowing for unequal variances).

3.6 Clinical attack rates (Chapter 5)

Clinical attack rates among districts reporting all three types of poliovirus were estimated based on the annual incidence of AFP associated with each poliovirus per 100,000 children under 5 years old, and per 100,000 children under 5 years old estimated to be unprotected by vaccination against the relevant serotype. For example, 10 AFP cases with WPV1 isolated reported from a population of 100,000 children with 80% vaccine-induced immunity against serotype 1 would give a clinical attack rate of 10 per 100,000 children or 50 per 100,000 susceptible children.

3.6.1 Clinical attack rate for poliomyelitis caused by each poliovirus

To estimate the clinical attack rate, incidence of reported cases of each of the three serotypes of poliomyelitis were calculated by year and district per 100,000 children aged under 5 years old. Total population numbers were taken from the 2006 census⁷⁷, scaled up or down for years after or before 2006 using a population growth rate of 2.38% per annum⁷⁷ and multiplied by the proportion of the population aged under 5 years old⁷⁶. Districts were only included that had reported at least one case of each type of paralytic polio during the study period to ensure comparison of areas infected by all three types of poliovirus. The mean clinical attack rate for each poliovirus was estimated for each district across years and then across all included districts, weighted by the populations in the districts⁷⁷. The variances in the clinical attack rates for each poliovirus type in each district were estimated across years to allow equal weighting for years 2005 to 2008 and half the weight of one year for 2009 (as we only include cases to

the mid-point of 2009 and assuming no seasonality and therefore equal attack rate throughout the year).

The variance across districts was then based on the Poisson assumption such that the variance is given by

$\sum_{d=1}^D \left(\frac{1}{Z}\right)^2 A_d$ where A_d is the attack rate in district d estimated across years as per the previous sentence

and $1/Z$ is the weighting given to that district depending on its population size. Confidence intervals were based on the Normal approximation to this distribution.

3.6.2 Clinical attack rate for poliomyelitis caused by each poliovirus per 100,000 'susceptible' children

To estimate the clinical attack rate per 100,000 'susceptible' children, the above method was repeated with the under 5 year old population numbers multiplied by the estimated proportion unprotected through direct vaccination in the relevant state. Therefore the attack rate here is equal to (number of cases reported)/(under 5 population * (1-proportion of the population protected through direct vaccination)).

Variances in the estimates are complicated by inclusion of the proportion of the population protected through direct vaccination. Bayesian confidence intervals were therefore calculated using the hierarchical model described above (3.3.3).

3.7 Estimation of hazard of reporting a cVDPV2 case (Chapter 5)

Monthly estimates of the hazard of a district reporting its first AFP case due to cVDPV2 were examined as a function of variables representing the force of infection from cases reported in the previous 6 months (Table 3.1), vaccine-induced population immunity, population size and population density. These were entered as time-varying covariates using a proportional hazards regression and the final model chosen on the basis of maximum likelihood as described further below. The proportional hazards assumption was assessed using a Schoenfeld global test⁸¹.

The hazard of a district reporting its first case of cVDPV2 was estimated using proportional hazards regression as follows

$$\ln[h(t)] = \ln[h_0(t)] + \eta_1 x_1 + \dots + \eta_p x_p$$

which can be written as

$$h(t) = h_0(t) \exp(\eta_1 x_1 + \dots + \eta_p x_p)$$

where $h(t)$ is the overall hazard (or risk) of a district reporting a case at time t , $h_0(t)$ is the baseline hazard, $x_1, \dots, x_p$ are explanatory covariates and $\eta_1, \dots, \eta_p$ are their associated coefficients. When maximising the likelihood of this function, $h_0(t)$ integrates out (as it is a constant) and from maximising the remaining partial likelihood we obtain the estimates of the coefficients $\eta_1, \dots, \eta_p$, which are the hazard ratios informing us of the impact of each variable on the overall hazard⁸².

The proportional hazards model allows the baseline hazard to vary but assumes that the coefficients are independent of time⁸². To assess the potential impact of a number of explanatory variables which changed over the course of the outbreak, we used a proportional hazards regression with time-varying covariates. The time period analysed was 26 May 2006 to 30 June 2009 as the second case had date of onset of 26 May 2006 (the first case was in July 2005 and as this was more than six months before the second case, it was excluded from the analysis). Each district formed a separate observation and time-varying covariates were updated monthly through the time period analysed resulting in 37 observations for each district. A sliding window of 6-months was used such that all covariates were estimated based on data in the six months leading up to the month in question.

Several different variables were calculated that attempt to describe the degree of exposure to cVDPV2, which we term the force of infection, and these variables were examined for their explanatory power.

Those based on physical distance from cases of cVDPV2 assumed that the location of a case was the centroid of the district of residence since more detailed location data are unavailable (the average area of a district is 1,200km²). Variables (Table 3.1) and their polynomials were entered into univariable proportional hazards models and the model with the maximum likelihood chosen.

Table 3.1 Variables examined describing the force of infection

Measure
Distance from the nearest case reported in the previous six months
Median distance from all cases reported in the previous six months
Mean distance from all cases reported in the previous six months
Number of cases reported in the previous six months within x km (where x was increased systematically by 1 until an optimal x was found which maximised the likelihood compared to all other x)
A “gravity” variable ⁸³ equal to $N_k \sum_j \frac{I_j}{d_{jk}}$ where k is the district in question, j represents other districts in the country, N is the population in district k , I is the incidence of cVDPV in each of the j districts in the previous six months and d is the distance between j and k .

Where median and mean distances were used, the first 9 cases and their associated time periods were excluded so that the median and mean distances were based on sufficient numbers. Therefore these analyses were based on data from September 06 instead of May 06.

Vaccine-induced population immunity levels were estimated as described above. After selection of a force of infection variable, immunity levels were entered into the model and this multivariable model compared with one without the immunity levels via a likelihood ratio test.

Similarly, the variables population and population density were assessed via likelihood ratio tests. Any confounding effects were also assessed and considered in selection of the final model.

3.8 Prediction of incidence of paralysis due to wild poliovirus type 1 (Chapter 6)

A negative binomial regression model was used to investigate risk factors for reporting WPV1 cases and to develop a model that could estimate the monthly number of WPV1 cases that a state would report. The dependent variable was the number of WPV1 cases reported by state. Since, in initial models, chi-squared values for all potential explanatory variables were found to be very high, a random intercept was used for each state. Where data over time were available, the explanatory variables represented the six months prior to the month in question. Table 3.2 shows the variables investigated and the time period they relate to. Data from July 2001 to June 2009 were modeled (since data from January 2001 were available and the previous six months data were used in the explanatory variables therefore modeling incidence started from July 2001).

Table 3.2 Potential explanatory variables investigated in the negative binomial regression models

Variable	Time period variable relates to
Proportion of <5 year old population reporting having received zero doses	Previous six months
Population immunity level (estimated as described in 3.3)	Previous six months
Proportion receiving 3 routine doses of tOPV by 52 weeks as reported in the DHS from 2003	One figure per state (no time variation) as described in 2.1
Proportion receiving 3 routine doses of tOPV by 52 weeks as reported in the NICS from 2006	One figure per state (no time variation) as described in 2.1
Proportion receiving 3 routine doses of tOPV by 52 weeks using data from the DHS for 2001 – 2004 and data from the NICS for 2005-2009	One figure per state for 2001-2004 and another for 2005-2009 as described in 2.1
Population size	As described in 2.1 and scaled up as described in 2.1 to provide monthly estimates of the population
Population density (i.e. population size / area of state in km ²)	Using population numbers as above and therefore available for previous six months
Number of non-polio AFP cases reported / population size	Previous six months
Number of WPV1 cases reported in bordering Nigerian states / population size	Previous six months
Number of WPV1 cases reported / population size	Previous six months

The number of WPV1 cases reported per month in each state was estimated using a negative binomial regression model as follows:

$$\ln[E(Y)] = a + \varphi_1 x_1 + \dots + \varphi_n x_n$$

where $E(Y)$ is the estimated number of WPV1 cases reported per month in each state, $x_1, \dots, x_n$ are explanatory covariates and $\varphi_1, \dots, \varphi_n$ are their associated coefficients. When maximising the likelihood of this function, a integrates out (as it is a constant) and from maximising the remaining partial likelihood we obtain the estimates of the coefficients $\varphi_1, \dots, \varphi_n$ which inform us of the impact of each variable on the overall estimate. The random variable y is assumed to follow a negative binomial distribution as follows:

$$P(Y = y) = \left(\frac{r}{(r + \lambda)} \right)^r \frac{\Gamma(r + y)}{\Gamma(y + 1)\Gamma(r)} \left(\frac{\lambda}{r + \lambda} \right)^y$$

where Γ is the gamma function. The mean of the negative binomial distribution is λ , the variance is $\lambda + \lambda^2/r$ and r is the overdispersion parameter⁸⁴.

All variables were logged (natural logarithm) before being entered into the model. Where variables could be equal to zero and therefore a natural logarithm does not exist, 0.5 was added to that variable for all observations to minimize bias in the covariates before logs were taken⁸⁵. Population size was automatically entered regardless of its significance although its coefficient was not forced to be equal to 1 (an “offset”) to allow the coefficient to vary as necessary with the data and the coefficient was examined for its proximity to 1. A coefficient of 1 would indicate that the incidence rate (number of cases/population size) was being modeled. Before building the model, the three variables on routine immunization were compared to decide which one would best represent the level of routine immunization across the country. Each of these three variables was separately entered into the negative binomial regression model with population size. The proportion of children receiving 3 routine doses of tOPV by 52 weeks of age as reported in the NICS from 2006 was highly significant ($p < 0.001$) while the other two

variables were non-significant. Therefore this variable was used throughout to represent the rate of routine immunization.

A backward elimination model selection procedure was used putting all variables into the model initially and then removing ones that were non-significant one by one. Each time a variable was removed the impact that removal had on other parameter estimates and standard errors was examined. If the removal of that variable had no substantial impact on other parameter estimates then it remained out of the model. Non-linear effects of the remaining variables were then investigated by entering centred squared transformations of each logged variable. All two-way interactions of linear variables were also tested in the model one by one. Significance of all non-linear and interaction terms were assessed conservatively using a Bonferroni correction where the significance level was equal to $0.05/n$ where n was the total number of non-linear and interaction terms investigated. Finally, any variables that were no longer significant following the inclusions of non-linear effects and interactions were removed (provided removal did not impact substantially upon other parameter estimates and the variables were not part of a significant interaction term).

The ability of the model to fit the observed number of WPV1 cases in a state was assessed visually by plotting the expected numbers of cases against those observed. We also assessed the ability of the model to detect high risk areas regardless of the actual predicted number of cases by examining percentiles. Let P_0 be the percentage of observations with 0 WPV1 cases (“low risk”), P_1 the percentage of observations with more than 0 and less than 11 cases (“medium risk”) and P_2 the percentage of observations with 11 or more cases (“high risk”). We then divided the expected WPV1 case numbers into three groups using P_0 , P_1 and P_2 (i.e. those in the bottom P_0 percent, those in the next P_1 percent and finally the top P_2 percent). We then calculated the percentage of state-months where the expected and observed risk groups were identical and where they differed.

4. The Effectiveness of Immunisation Against Paralytic Poliomyelitis in Nigeria

4.1 Summary

Background Paralytic polio cases in Nigeria declined in 2007 following the introduction of newly licensed monovalent oral poliovirus vaccines and new vaccine delivery tactics. Understanding the relative contribution of these vaccines and improved coverage is essential to future planning.

Methods The field efficacies of mOPV1 and tOPV were estimated from the reported number of doses received by polio cases and matched controls identified in the national surveillance database of 27,379 acute flaccid paralysis cases from 2001 to 2007. Vaccine coverage and vaccine-induced immunity were estimated from reporting of doses received by children in the database with paralysis not caused by polio.

Results The estimated efficacies of mOPV1 and tOPV against type 1 polio were 67% (95% CI: 39-82%) and 16% (10-21%) per dose respectively, and of tOPV against type 3 polio was 18% per dose (9-26%). In the North West of Nigeria, which has reported the majority of cases during the study period, coverage with at least 1 dose of vaccine increased from 59% to 78% and vaccine-induced immunity levels among children under 5 years old more than doubled to 56% between 2005 and 2007.

Conclusions The 4-fold higher efficacy of mOPV1 and moderate gains in coverage dramatically increased vaccine-induced immunity against serotype 1 in northern states. Further increases in coverage in infected states are required to achieve the levels of vaccine-induced immunity associated with sustained elimination in other parts of the country.

Note: A modified version of this chapter has been published as: Jenkins HE, Aylward RB, Gasasira A, et al. Effectiveness of immunization against paralytic poliomyelitis in Nigeria. *The New England Journal of Medicine* 2008;359:1666-74. (See publications by the candidate, Appendix).

4.2 Introduction

By 2003, the GPEI had interrupted the transmission of indigenous wild polioviruses in all but 6 countries worldwide, including Nigeria⁸⁶. In that year, however, the temporary suspension of all polio immunization in one Nigerian state contributed to a national epidemic of polio and the re-infection of at least 20 previously polio-free countries⁵⁸. Although polio immunization was reinstituted within 12 months, coverage remained low across northern Nigeria and case numbers continued increasing.

In early 2006, Nigerian health authorities adopted new tools and tactics to accelerate polio eradication. To respond to lingering community concerns, 'Immunization Plus Days' (IPDs) replaced NIDs, offering a range of childhood vaccinations and other health interventions alongside OPV. To enhance the impact of each immunization contact against the most prevalent poliovirus serotype, tOPV was therefore replaced with mOPV1 and mOPV3 in a number of IPDs from February 2006 and July 2007 respectively²⁷⁻²⁹. In 2007, WPV1 cases in Nigeria fell by 86% compared to 2006, contributing to an overall decline in WPV cases of 75% (Appendix Tables A1a-b). International exportations of poliovirus also plummeted, suggesting a vast improvement in the prospects for global eradication.

Although the combination of IPDs and mOPV1 is generally credited with the 2007 progress, a critical evaluation of the interventions used is essential to assessing the feasibility of completing polio elimination in Nigeria and, ultimately, the world. An estimate of the efficacy of these vaccines is especially important in the context of the recent resurgence of WPV1 cases in 2008 in northern Nigeria. We investigated the protective efficacies of mOPV1 and tOPV in Nigeria, the changes in vaccine coverage with the introduction of the IPD strategy, the resulting vaccine-induced population immunity over time, and the levels of immunity associated with local interruption of wild poliovirus in this setting. We discuss the implications of these results for the elimination of polio from Nigeria.

4.2 Results

4.2.1 Vaccine efficacy

A total of 2532 WPV1 and 1092 WPV3 cases were reported during the study period. Of these, 1174 (46%) WPV1 cases and 502 (46%) WPV3 cases were matched with a suitable control (Table 4.1). The estimated efficacy of tOPV against paralysis from WPV1 across Nigeria was 16% per dose (95% confidence interval (CI): 10-21%) and of mOPV1, 67% per dose (95% CI: 39-82%). The estimated efficacy of tOPV against paralysis from WPV3 was 18% per dose (95% CI: 9-26%). There was insufficient use of mOPV3 to allow the efficacy of this vaccine to be estimated reliably.

Table 4.1 Estimated protective efficacy against WPV1 and WPV3 polio per dose of the oral poliovirus vaccines used in Nigeria, 2001- 2007

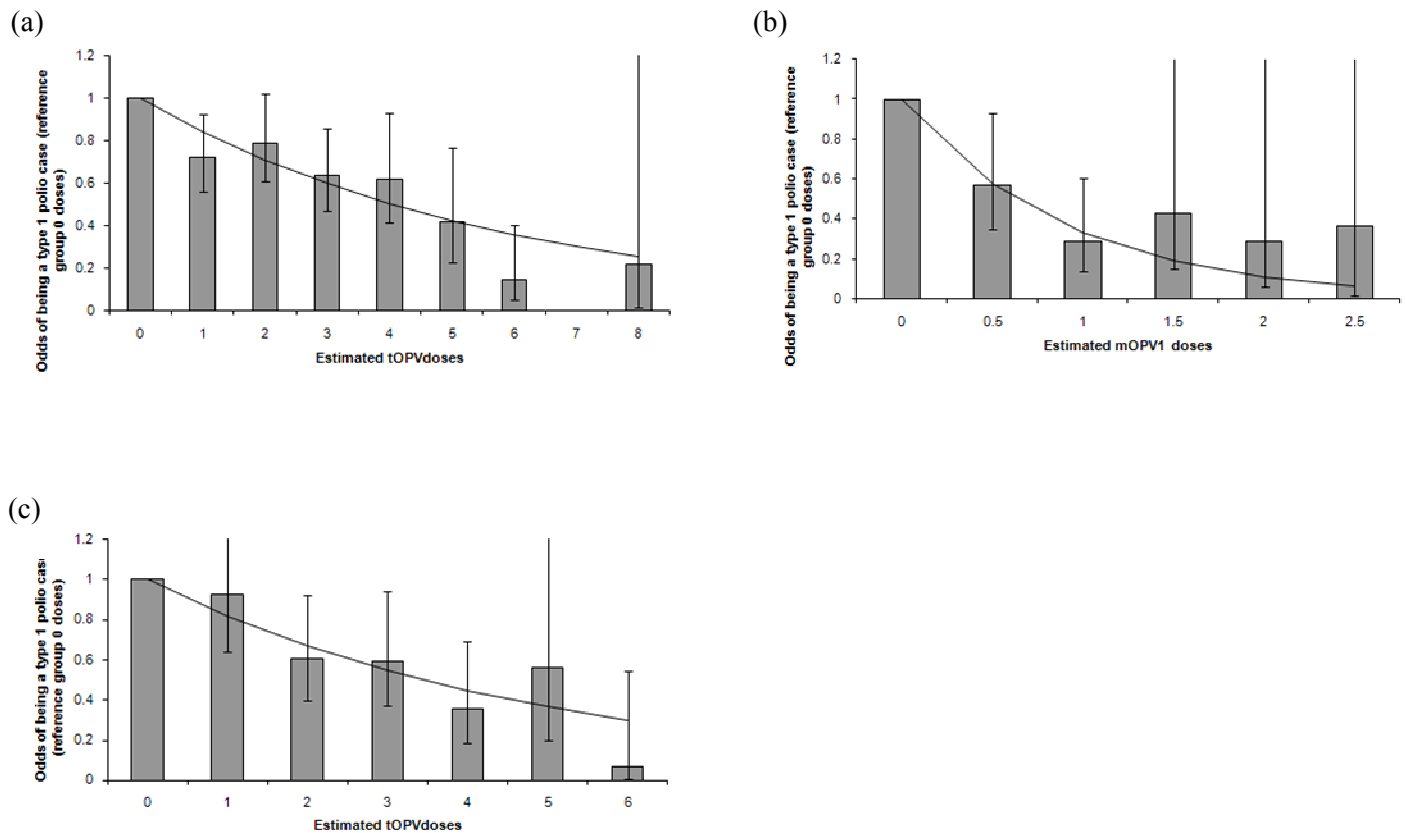
Location	WPV1				WPV3		
	Cases	Case-control	mOPV1 efficacy	tOPV efficacy	Cases	Case-control	tOPV efficacy
		matches, n	(%) (95% CI)	(%) (95% CI)		matches, n	(%) (95% CI)
		(% of cases)				(% of cases)	
North West	1899	925 (49%)	63 (27, 82)	13 (6, 19)	851	402 (47%)	17 (7, 26)
North East	437	158 (36%)	58 (0, 88)	23 (7, 37)	173	67 (39%)	18 (0, 40)
North	154	68 (44%)	100 (0, 100)	23 (1, 40)	52	16 (31%)	24 (0, 56)
Central							
South**	42	23 (55%)	N/A*	54 (4, 78)	16	8 (50%)	12 (0, 54)
Overall	2532	1174 (46%)	67 (39, 82)	16 (10, 21)	1092	502 (46%)	18 (9, 26)

*There were no reported cases of WPV1 in the South since the introduction of mOPV1 to the end of the study period

**Results for the South include all three southern zones (South West, South South and South East)

There were no significant differences between vaccine efficacies across the country (Table 4.1) or when estimated separately by year or age (Appendix Tables A3a-b). There was no evidence of an interaction between mOPV1 and tOPV in the WPV1 model ($p=0.82$). Comparisons of the fit of the model with a constant efficacy per dose of vaccine with a model that allowed the protective efficacy to vary with dose number showed that a constant efficacy per dose was a reasonable assumption for both mOPV1 and tOPV, consistent with the all-or-nothing mode of action of these live virus vaccines ($p=0.93$ and $p=0.085$ respectively against WPV1 and $p=0.36$ for tOPV against WPV3) (Figure 4.1). Tighter matching of cases and controls by age and date at onset of paralysis did not substantially alter the estimates of vaccine efficacy (Appendix Table A4). Sensitivity analyses showed that our results were robust to varying assumptions regarding the number of doses received via routine immunization (Appendix Table A5).

Figure 4.1 Odds of being a WPV1 case by estimated number of doses received of: (a) tOPV (only up to 8 doses shown), (b) mOPV1 (only up to 2.5 doses shown). Odds of being a WPV3 case by number of doses received of: (c) tOPV (only up to 6 doses shown). Error bars indicate 95% confidence intervals. The curved line represents the relationship between odds of being a case and doses when a log linear relationship is assumed.



The estimated sensitivity of laboratory testing for wild poliovirus was 99% and 98% for WPV1 and WPV3 respectively. The prevalence of type 1 and 3 poliovirus among AFP cases was estimated at 9.3% and 3.9% respectively. Therefore, the probability of misclassifying a non-polio AFP case as WPV1 or WPV3 was 0.0011 and 0.0006 respectively.

4.2.2 Immunization coverage and program effectiveness

Significant improvements in immunization coverage were registered between 2005 (pre-mOPV1 and IPDs) and 2007 (post- mOPV1 and IPDs) in the zones which have reported the majority of polio cases (North West and North East) ($p < 0.001$; Table 4.2). Coverage in the South East declined significantly between 2005 and 2007 ($p < 0.001$; Table 4.2). Coverage in all other zones is high and there were no significant changes between 2005 and 2007.

Table 4.2 Percentage of non-polio AFP cases (under the age of 5 years) receiving either 0, between 1 and 3 or more than 3 doses (regardless of vaccine type) and resulting percentage of the under 5 population protected against paralysis from WPV1. Figures are for 2005 and 2007.

Year	Number of reported doses	North West* (%)	North East* (%)	North Central (%)	South West (%)	South East* (%)	South South (%)	Whole country* (%)
2005	0	41	28	4	2	2	1	15
	1-3	44	42	30	28	39	31	37
	>3	15	30	66	70	59	69	47
Percentage protected by vaccination % (95% CI)		22 (15, 28)	31 (28, 35)	49 (46, 52)	56 (51, 62)	50 (45, 54)	53 (49, 57)	42 (40, 44)
Sample size in 2005		909	358	529	480	219	229	2724
2007	0	21	9	3	1	7	3	9
	1-3	55	59	38	27	46	31	43
	>3	24	32	59	72	46	66	48
Percentage protected by vaccination % (95% CI)		56 (41, 71)	61 (48, 73)	65 (53, 77)	83 (72, 94)	71 (57, 84)	81 (72, 91)	69 (62, 76)
Sample size in 2007		740	318	522	450	273	424	2727

*Distribution of doses between 0, 1-3 and >3 doses was significantly different between 2005 and 2007 in these zones (p<0.001 in all three zones and in the entire country)

The combination of improved coverage and use of the more efficacious mOPV1 has resulted in substantial increases in the proportion of children protected through direct immunization against paralysis from WPV1 (Figure 4.2 and Table 4.2). In the North West 33% of the improvement in vaccine-induced immunity between 2005 and 2007 was due to improved coverage, whilst in the south of the country the improvement in vaccine-induced immunity was solely due to the introduction of the monovalent vaccine (Appendix Tables A6 and A7 and Table 4.3).

Table 4.3 Estimated population immunity levels from direct vaccination against paralysis from WPV1 (shown as percentage of the population under 5 years old that are protected through direct vaccination), 2005-7 calculated assuming that all SIA used tOPV. Also shown are the resulting changes in immunity due to coverage changes, the actual changes seen 2005-7 (from data in Appendix Table A6) and therefore the fraction of the improvement in vaccine-induced immunity that resulted from the introduction of mOPV1

Zone	<u>Assuming no mOPV1</u>				Estimated total change from 2005 to 2007	Improvement due to use of mOPV1 (% of total change)
	2005 (%)	Year 2006 (%)	2007 (%)	Change from 2005 to 2007 due to coverage changes		
North West	22	25	33	11	33	22 (67)
North East	31	37	37	6	29	23 (79)
North Central	49	51	49	0	15	15 (100)
South West	56	57	56	0	25	25 (100)
South South	53	55	50	-3	26	29 (100)
South East	50	48	41	-9	17	26 (100)

The estimated fraction of children protected against WPV1 strongly correlates with reporting of WPV1 cases (Figure 4.2). Correlation was statistically significant in all years, rising significantly in more recent years ($p=0.023$; Table 4.4). Calculating the average annual WPV1 incidence per state as a function of the fraction of children under 5 years old protected against WPV1 suggests that in some areas a level of 80% protected has been sufficient to achieve local elimination (Figure 4.3).

Figure 4.2 Maps of Nigeria from 2001 to 2007 with state boundaries (thin black lines) marked. States are shaded according to proportion of the population protected by vaccination against paralysis from WPV1 (to represent vaccine coverage) (see legend). Black dots represent locations of a reported case of paralysis from WPV1. (a) shows the boundaries of the 6 administrative zones.

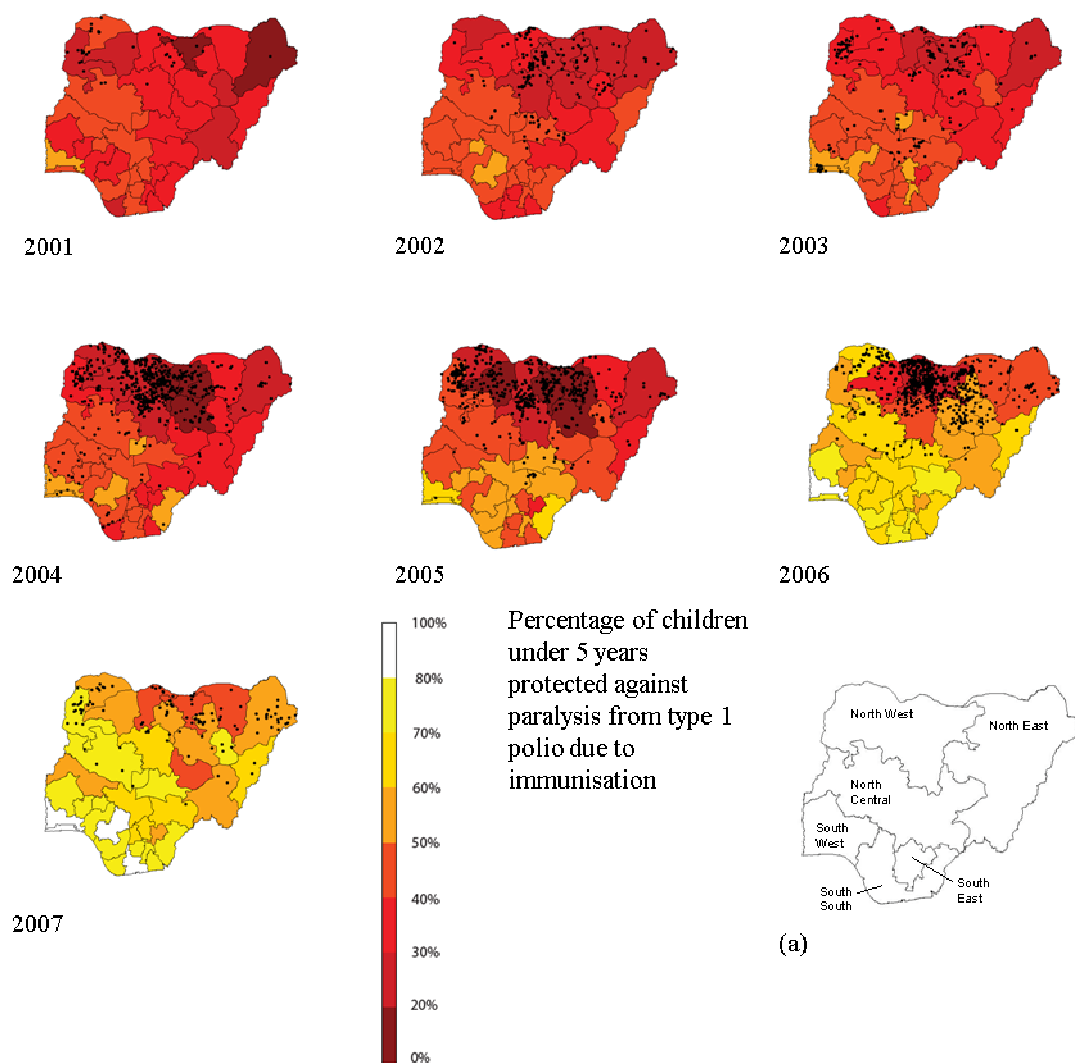
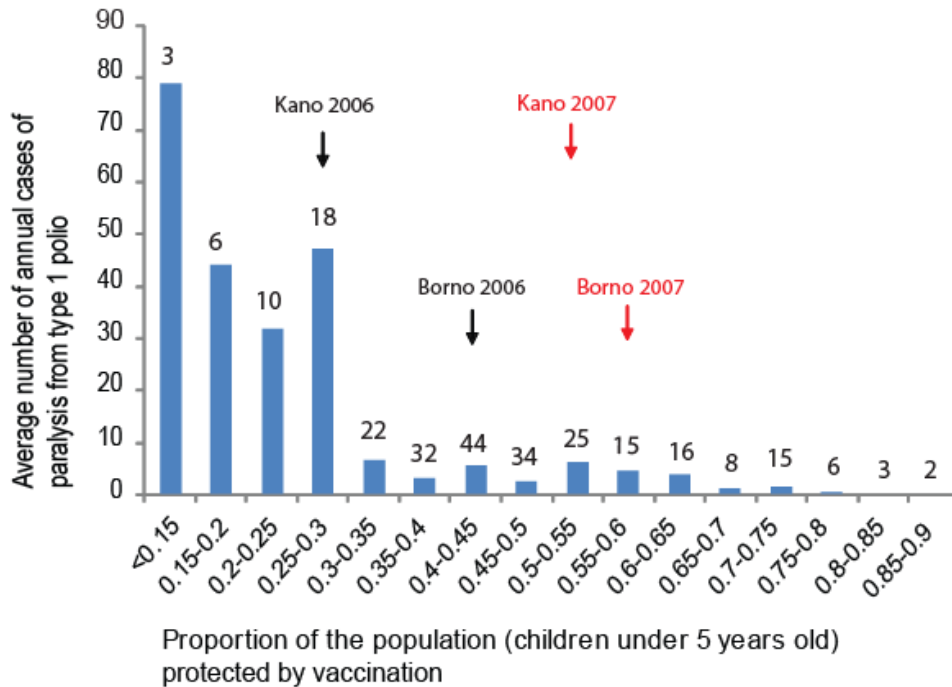


Table 4.4 Spearman's rank correlation coefficient, by year, measuring correlation between the proportions of the population (under 5 years) protected by vaccination and incidence of reported WPV1 cases. Data are grouped by state of residence.

Year	Spearman's rank correlation coefficient (95% CI)	P-value	Spearman's rank correlation coefficient (95% CI)
2001	0.43 (0.12, 0.66)	0.007	2001-3 combined: 0.38 (0.21, 0.53)*
2002	0.51 (0.22, 0.71)	0.001	
2003	0.39 (0.07, 0.63)	0.016	
2004	0.62 (0.36, 0.78)	<0.001	2004-7 combined: 0.61 (0.49, 0.70)*
2005	0.71 (0.50, 0.84)	<0.001	
2006	0.75 (0.55, 0.86)	<0.001	
2007	0.54 (0.26, 0.73)	<0.001	

*Correlation post-2003 significantly higher than in earlier years ($p = 0.023$).

Figure 4.3 Average number of annual WPV1 cases per state by the proportion of the population (children under 5 years old) protected through direct vaccination. Number of observations included in each bar is indicated above the bar. Positions (in terms of proportion of the population protected) of two key states are shown for 2006 and 2007.



4.3 Discussion

This study provides the first estimates of the efficacy of OPVs in Nigeria and the impact of these vaccines on population immunity. Although tOPV was successfully used to interrupt transmission of indigenous WPV1 across southern Nigeria, the recently licensed mOPV1, with an estimated efficacy per dose that is 4-fold higher than that of tOPV, substantially improves the prospects for accelerating elimination in northern Nigeria. Due to improvements in immunization coverage and the introduction of mOPV1, vaccine-induced immunity in the North West and North East zones (which reported 96% of WPV1 cases

in 2006) more than doubled between 2005 and 2007. However, further gains in immunization coverage are required to achieve elimination, as 21% of children in the North West zone (where the majority of cases have occurred) still report never having received a single dose of vaccine, with a further 55% having received fewer than the recommended 4 doses.

The estimated efficacy of tOPV in Nigeria is similar to that found in India, with the exception of the northern Indian state of Uttar Pradesh where significantly lower efficacy of OPVs appears to be the result of a high prevalence of diarrhea and other infections that interfere with seroconversion⁷. The efficacy of tOPV did not show statistically significant variability across Nigeria, although case numbers in the south were limited (Table 4.1). The efficacy of mOPV1 in northern Nigeria is higher than in Uttar Pradesh, India⁴⁸ and is comparable to rates of seroconversion following mOPV1 administration in other developing countries⁴⁹. This suggests that in contrast to northern India, the persistence of poliovirus in the north of Nigeria is not a result of environmental conditions that can compromise vaccine efficacy.

The precision of our vaccine efficacy estimates could be affected by the accuracy of the reporting of OPV doses and potential differences in exposure to poliovirus between cases and controls. However, the double blinded nature of the parent/carer interviews to the polio status of the child minimizes the risk of systematic bias in dose reporting. Also, controls came from the same area as cases and so are likely to have had similar exposure to virus, and indeed efficacy estimates were robust to tighter matching of cases with controls. Nearly 50% of cases were matched to a control and the resulting exclusion of some cases does not appear to have introduced any bias (Appendix Tables A8a-b). Perhaps more importantly, irrespective of the precision of the point estimate of vaccine efficacy, the relative gain calculated for the efficacy of mOPV1 versus tOPV against WPV1 is robust to these potential biases. Vaccine quality is

unlikely to be an issue, since temperature sensitive vaccine vial monitors have been used nationwide since 2000 to prevent the administration of vaccine with low potency.

Our estimates of population immunity do not account for secondary transmission of vaccine virus, maternal antibodies in the first few months of life or natural immunity from exposure to circulating wild poliovirus. However, they allow a direct comparison of vaccine-induced immunity among states that have had SIAs with different types of vaccine and variable frequency and coverage, thereby providing a good relative indication of program effectiveness. The improvements observed in vaccine coverage illustrate that increases in immunity levels in North West Nigeria are not purely a result of higher efficacy of mOPV1. Many factors are likely to have contributed, including efforts by field staff and local and national leaders to address the concerns of community leaders and parents that contributed to the 2003 suspension of OPV use, including the introduction of IPDs.

The encouraging findings of this study must be interpreted with caution when assessing the overall outlook for elimination in Nigeria. With substantial population movements and an annual birth rate of around 40 per 1000 in the population⁷⁶, growth in the susceptible population is considerable. Figure 4.3 suggests that while a level of direct immunity from vaccination of around 30% can reduce WPV1 transmission in Nigeria, a level of at least 80% is required for sustained local elimination of transmission. As of 2007, some control appears to have been achieved in many northern states, but considerable further improvements are still required to reach a level which may eliminate polio (Figure 4.2). The consequences of such ongoing immunity gaps were evident in the emergence of a cVDPV in this area⁸⁷ and a resurgence of WPV1 cases in early 2008² which have spread to the South. However, giving each child in the North West just one additional dose of mOPV1 could increase direct population immunity to over 80% in much of this area – a substantial increase from the 56% recorded in 2007.

India and Nigeria reported 88% of polio cases worldwide in 2007 and now hold the key to global eradication. In contrast to India, vaccine coverage is now clearly still the main barrier to completing polio elimination in northern Nigeria. However, the relatively high efficacy of mOPV1 in Nigeria provides the potential for rapidly eradicating WPV1, provided that vaccination coverage continues to improve in the north of the country.

5. Implications of a circulating vaccine-derived poliovirus in Nigeria for polio eradication

5.1 Summary

Background The largest recorded outbreak of a cVDPV to be observed whilst transmission was ongoing, detected in Nigeria, provides a unique opportunity to analyze the pathogenicity, clinical severity and effectiveness of control measures for cVDPVs relative to WPV.

Methods Cases of paralysis due to cVDPV2, WPV1 and WPV3 in Nigeria were identified from 1 January 2005 to 30 June 2009. The clinical characteristics of these cases, the clinical attack rates for each virus, and the efficacies of OPVs against paralysis by the cVDPV and WPVs were compared.

Results No significant differences were found between the clinical severity of the 278, 2323 and 1059 AFP cases with cVDPV2, WPV1 and WPV3 respectively in their stool. The estimated average annual clinical attack rates of WPV1, cVDPV2 and WPV3 were 6.8 (95% confidence interval: 5.9-7.6), 2.7 (1.9-3.6) and 4.0 (3.4-4.7) per 100,000 susceptible children respectively. The estimated efficacy of tOPV against paralysis by cVDPV2 was 38% (15-54%) per dose, substantially higher than that against paralysis by WPV1 (13% (8-18%)) or WPV3 (20% (12-26%)). More frequent use of serotype 1 and 3 mOPVs has resulted in improvements in vaccine-induced population immunity against these serotypes and declines in immunity to cVDPV2.

Conclusions The attack rate and severity of disease associated with this cVDPV are similar to those of WPV. International planning for the management of WPV risks both pre- and post-eradication must encompass scenarios under which equally virulent and pathogenic cVDPVs could emerge.

Note: A modified version of this chapter has been published as: Jenkins HE, Aylward RB, Gasasira A, et al. Implications of a circulating vaccine-derived poliovirus in Nigeria. *The New England Journal of Medicine* 2010;362(25):2360-9. (See publications by the candidate, Appendix).

5.2 Introduction

Since 1988, when the World Health Assembly resolved to eradicate poliovirus, annual incidence of paralytic poliomyelitis has fallen by more than 99%. However, the annual number of cases has remained relatively constant between 2003 and 2009 and continued transmission in endemic countries, especially Nigeria, led to a resurgence of re-infected countries across Africa in 2008-9^{2, 88}. The emergence of a cVDPV2 has complicated the epidemiology of polio in Nigeria as well as vaccine selection and scheduling for SIAs⁸⁷.

A cVDPV is defined by the occurrence of 2 or more AFP cases with closely related vaccine-derived poliovirus and occurs when a vaccine (Sabin) poliovirus increases in neurovirulence and transmissibility following mutation². cVDPVs have usually recombined with other enteroviruses and their spread is associated with inadequate vaccine coverage³⁸. Globally 12 independent cVDPV outbreaks have been identified to date^{2, 38, 39}. However, little is known about their clinical features or the risks they pose to eradication. Consequently, their significance has been much debated with some arguing cVDPVs mark the death knell for eradication^{52, 89}, whilst others have suggested that they represent a minor risk and/or cause only mild illness⁴².

Serotype 2 cVDPVs are particularly concerning because serotype 2 WPV (WPV2) is considered to have been eradicated⁴⁶. The ongoing outbreak of paralytic disease associated with this cVDPV2 in Nigeria is unique in being the largest to date, occurring in the presence of extensive transmission of indigenous WPV1 and WPV3 circulation and having been confirmed whilst transmission is occurring. Therefore, control measures with tOPV, the only vaccine currently licensed in Nigeria that protects against serotype 2, have been interspersed with aggressive use of the more efficacious mOPVs to limit the national and international spread of WPVs^{48, 49, 75, 90}.

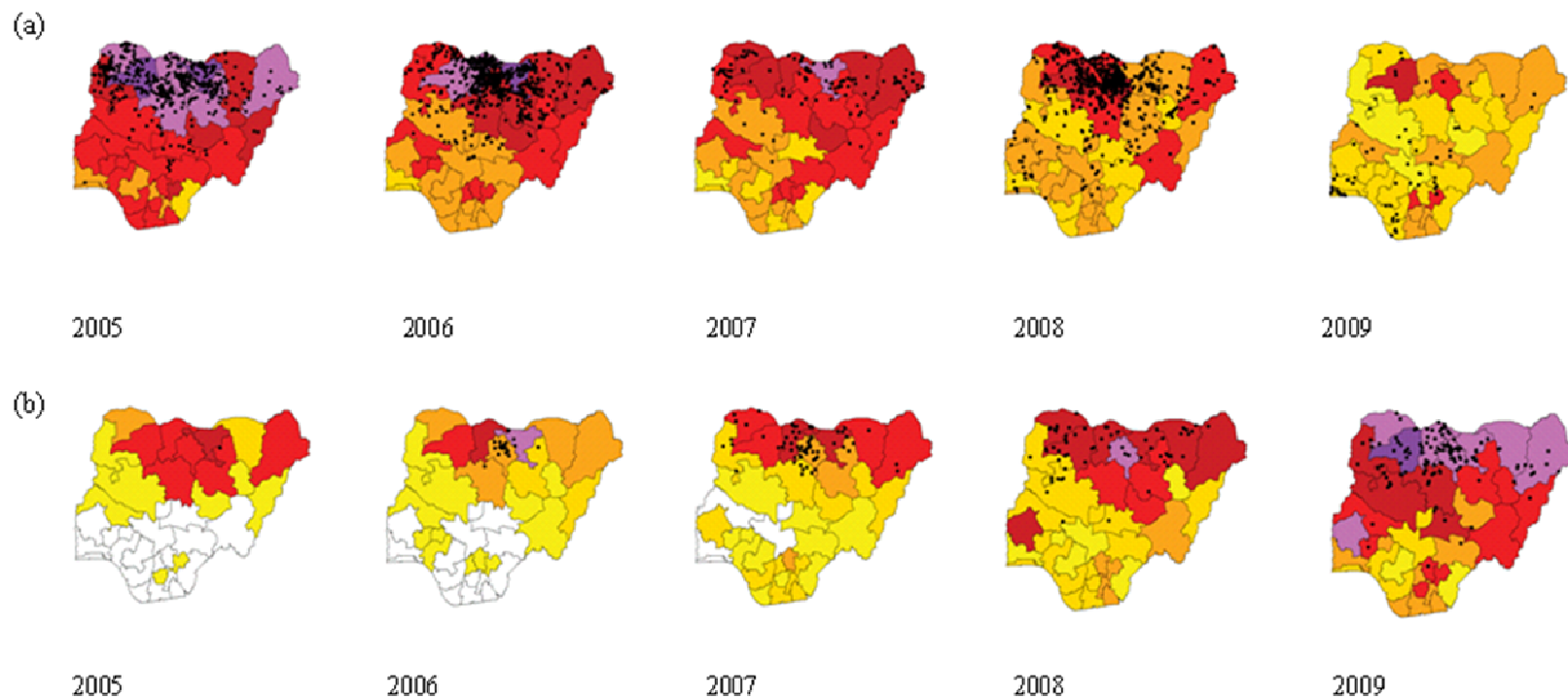
Despite both large- and small-scale tOPV campaigns in 2006-8, numbers of AFP cases associated with cVDPV2 increased in early 2009. Optimizing the balance of tOPV, mOPVs and the recently developed bivalent OPV (serotypes 1 and 3) (bOPV)⁹¹ in upcoming SIAs in Nigeria, and appropriately planning for post-eradication risk management, requires a deeper understanding of the pathogenicity and clinical severity of paralytic polio caused by cVDPVs relative to WPVs as well as the effectiveness of current control strategies.

5.3 Results

5.3.1 Severity of disease and clinical attack rates

A total of 23,004 AFP cases were reported over the study period, of which 278 had cVDPV2 in their stool, 2,323 WPV1, 1,059 WPV3 and 2 both WPV1 and WPV3 (Figure 5.1, Figure 3.2, Appendix Tables A1a-c). cVDPV2 cases were broadly similar to those associated with WPV1 and WPV3 in terms of their age and sex distribution. There were no significant differences in the severity of clinical disease (Table 5.1, Appendix Table A9).

Figure 5.1 Estimated proportion of children aged under 5 years with vaccine-induced immunity against paralysis by (a) WPV1, (b) cVDPV2 and (c) WPV3 by state during 2005, 2006, 2007, 2008 and Jan-June 2009. Black dots indicate locations of AFP cases with the relevant poliovirus type reported in that year.



(c)

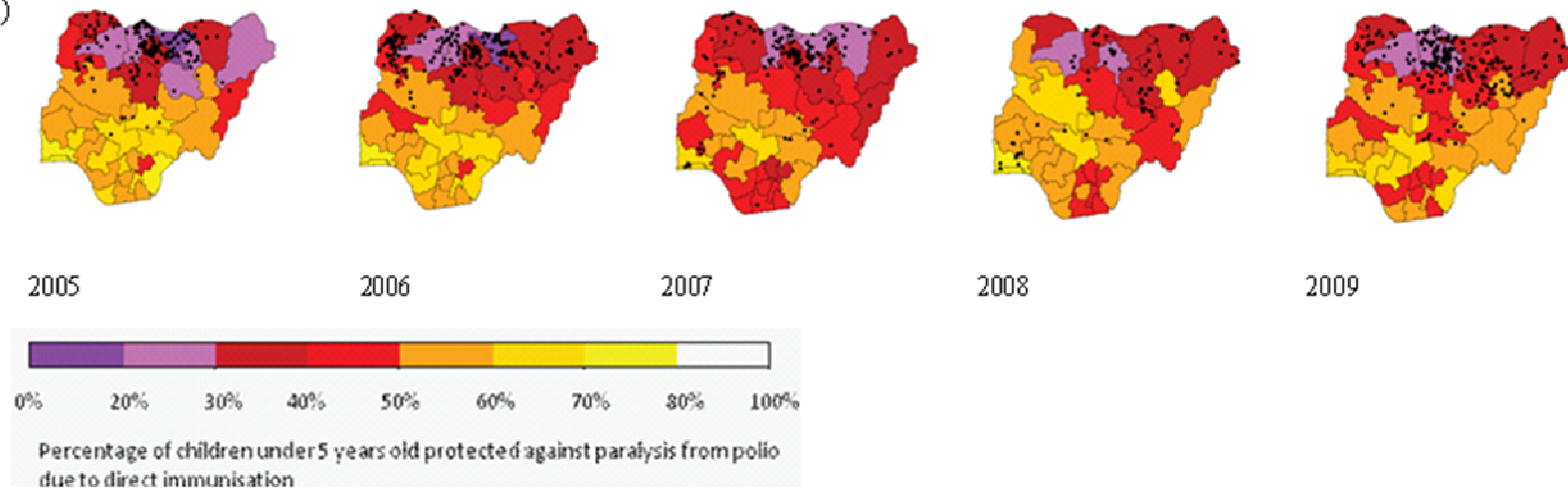


Table 5.1 Characteristics of AFP cases with WPV1, cVDPV2 or WPV3 isolated from their stool

Variable		WPV1 (n=2323)	cVDPV2 (n=278)	WPV3 (n=1059)	P-value (cVDPV2 vs. WPV1) [†]	P-value (cVDPV2 vs. WPV3) [†]
Sex, n (%)	Male	1312 (56)	172 (62)	609 (58)	0.04	0.11
	Female	1010 (43)	101 (36)	449 (42)		
	Missing	1 (<1)	5 (2)	1 (<1)		
Age (yrs), mean (SE)		2.36 (0.03)	2.57 (0.10)	2.44 (0.05)	0.04	0.74
	Missing, n (%)	255 (11)	35 (13)	87 (8)		
Asymmetrical paralysis	Yes, n (%)	1719 (74)	209 (75)	779 (74)	0.37	0.34
	No, n (%)	570 (25)	60 (22)	265 (25)		
	Missing, n (%)	34 (1)	4 (1)	13 (1)		
Progression to paralysis within 3 days	Yes, n (%)	2278 (98)	269 (97)	1038 (98)	>0.99	>0.99
	No, n (%)	25 (1)	3 (1)	13 (1)		
	Missing, n (%)	20 (1)	6 (2)	8 (1)		
Fever at onset	Yes, n (%)	2286 (98)	269 (97)	1039 (98)	>0.99	0.75
	No, n (%)	18 (1)	2 (1)	13 (1)		
	Missing, n (%)	19 (1)	7 (3)	7 (1)		
Number of limbs affected	0, n (%)	11 (<1)	0 (0)	9 (1)	0.06	0.17
	1, n (%)	1197 (52)	142 (51)	567 (54)		
	2, n (%)	996 (43)	104 (37)	423 (40)		
	3, n (%)	68 (3)	10 (4)	35 (3)		
	4, n (%)	44 (2)	12 (4)	23 (2)		
	Missing, n (%)	7 (<1)	10 (4)	2 (<1)		

Residual	Yes, n (%)	517 (22)	52 (19)	240 (23)		
paralysis at	No, n (%)	76 (3)	9 (3)	37 (2)		
follow up	Lost to follow up, n (%)	2 (<1)	0 (0)	1 (<1)	0.69	0.84
	Dead at follow up, n (%)	4 (<1)	0 (0)	4 (<1)		
	Missing, n (%)	1724 (74)	217 (78)	757 (71)		

†Tests exclude observations with missing data and those lost or dead at follow up

In areas reporting all three types of poliovirus the estimated clinical attack rates (as measured by annual incidence) of paralytic polio were 6.8, 2.7 and 4.0 per 100,000 susceptible children under 5 years for WPV1, cVDPV2 and WPV3 respectively (Table 5.2).

Table 5.2 Estimated per dose efficacies of oral polio vaccines against paralysis by the different polioviruses circulating in Nigeria and the estimated annual clinical attack rates.

Variable	WPV1 (n=2,323)	cVDPV2 (n=278)	WPV3 (n=1,059)
Per dose vaccine efficacy (%; 95% Confidence Interval)			
Number of cases matched to controls (% of total cases)	1739 (75%)	184 (66%)	812 (77%)
mOPV1	43 (33, 51)	N/A*	N/A*
tOPV	13 (8, 18)	38 (15, 54)	20 (12, 26)
mOPV3	N/A*	N/A*	53 (16, 74)
Annual clinical attack rate			
Number of cases aged <5 years**	1,075	211	508
Per 100,000 susceptible children under 5 years old (95% Confidence Interval)	6.8 (5.9, 7.7)	2.7 (1.9, 3.6)	4.0 (3.4, 4.7)
Per 100,000 children under 5 years old (susceptible and vaccinated combined) (95% Confidence Interval)	5.3 (4.9, 5.6)	1.5 (1.3, 1.6)	3.2 (2.8, 3.5)

*Not estimated here since this vaccine is not used to target this type of polio (see Appendix Table A10)

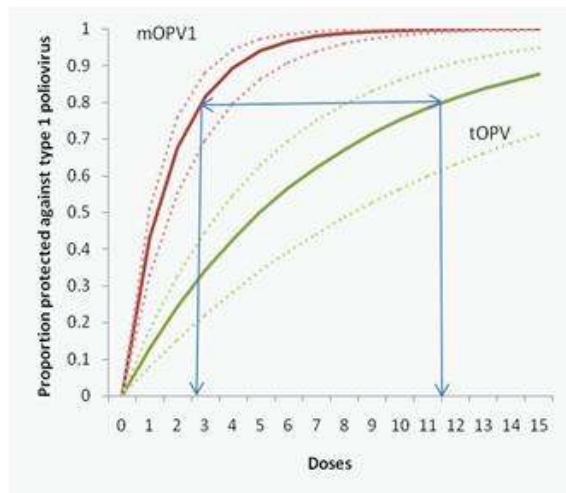
**In districts that have reported AFP cases resulting from all three poliovirus types during the study period

5.3.2 Vaccine efficacy

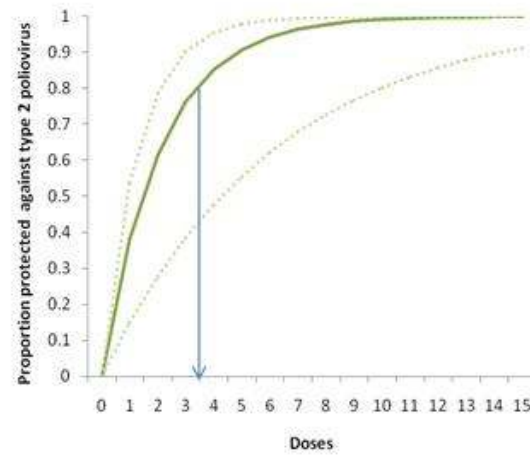
The estimated efficacy per dose of tOPV against paralysis by cVDPV2 was greater than that against paralysis by WPV1 ($p=0.04$) and WPV3 ($p=0.12$) (Table 5.2; Figure 5.2). mOPV1 was significantly more protective than tOPV against paralysis by WPV1 ($p<0.001$) and mOPV3 was non-significantly more protective than tOPV against paralysis by WPV3 ($p=0.08$). There were no significant effects of any OPVs against serotypes towards which the OPVs are not targeted (Appendix Table 10) although there was a borderline significant protection by mOPV3 against paralysis by WPV1.

Figure 5.2 The predicted proportion of children protected against paralysis from (a) WPV1, (b) cVDPV2 and (c) WPV3 by OPV type plotted against the number of doses, based on the estimated efficacy per dose. Solid red lines indicate monovalent vaccines and solid green lines, the trivalent vaccine. Dotted lines indicate 95% confidence intervals. Number of doses required for 80% protection are shown for illustration and for comparison of the monovalent vaccine with the trivalent vaccine.

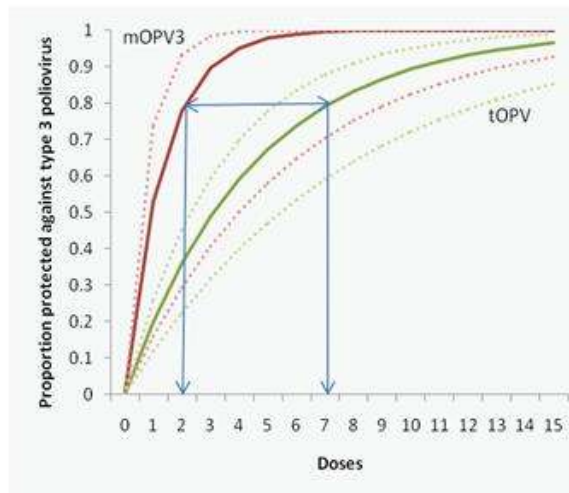
(a)



(b)

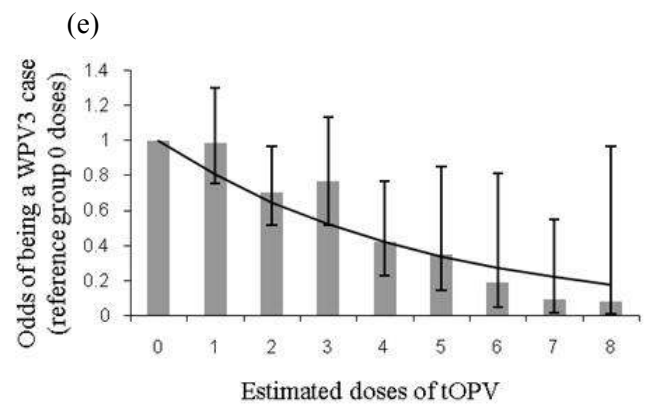
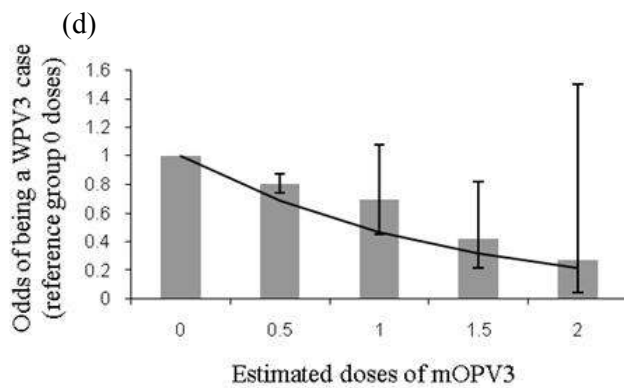
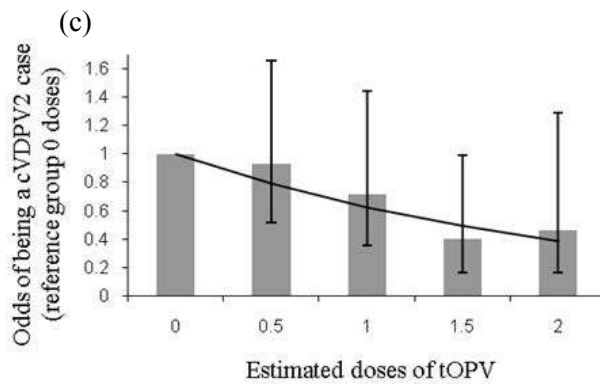
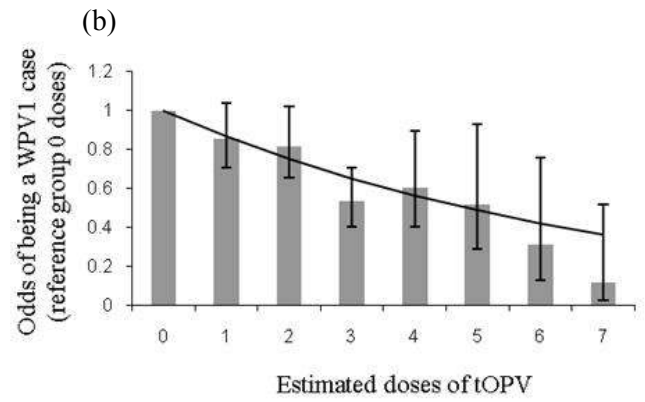
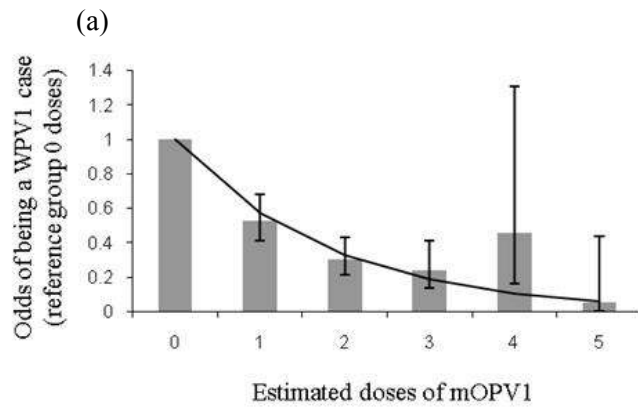


(c)



A regression model with a variable efficacy per dose of OPV did not provide a significantly better fit than a model with constant efficacy per dose (likelihood ratio test $p > 0.2$ for all vaccine and poliovirus types; Figure 5.3).

Figure 5.3 Relative odds of being a paralytic polio case by estimated number of OPV doses received. The estimate relative odds are given by the bars, with error bars indicating 95% confidence intervals, and the curved line represents the relationship between the relative odds of being a case and number of doses based on a constant vaccine efficacy per dose received. (a) Relative odds of AFP associated with WPV1 by estimated number of mOPV1 doses received (up to 5 doses shown), (b) relative odds of AFP associated with WPV1 by estimated number of tOPV doses received (up to 7 doses shown), (c) relative odds of AFP associated with cVDPV2 by estimated number of tOPV doses received (due to small numbers of doses received, half doses are shown, up to 2.5 doses), (d) relative odds of AFP associated with WPV3 by estimated number of mOPV3 doses received (due to small numbers of doses received, half doses are shown, up to 2 doses), (e) relative odds of AFP associated with WPV3 by estimated number of tOPV doses received (up to 5 doses shown)



The majority of cases were matched to a suitable control. There were no substantial differences in vaccine efficacy across the country or when estimated separately by year or age. Where some significant interactions were noted (for serotype 1 only), matching on district instead of state resolved this bias

resulting from SIAs carried out at a district (not state) level and overall estimates of vaccine efficacy were unchanged (Appendix Tables A11a-b). Tighter matching (by age, date and location) of cases and controls did not substantially alter the estimates of vaccine efficacy (data not shown). Although matching on district instead of state increased the estimate of vaccine efficacy against cVDPV2 to 54% per dose, only 21% of cases were matched leading to wide confidence intervals. Hence matching by state was preferable although it may have slightly underestimated the overall vaccine efficacy.

Sensitivity analyses showed that our results were reasonably robust to varying assumptions regarding the number of doses received via routine immunization and that tOPV was consistently more efficacious against paralysis by cVDPV2 than WPV1 or WPV3 (Appendix Tables A12a-c).

5.3.3 Vaccine-induced population immunity

Estimated vaccine-induced population immunity to WPV1 poliomyelitis increased significantly in all zones from 2005 to 2009 ($p < 0.02$), particularly in the North West and North East zones (+7.4% and +8.1% respectively annually; Figure 5.1, Tables 5.3a-c). However, estimated vaccine-induced population immunity to cVDPV2 poliomyelitis declined significantly in the study period in all zones (range: -4.7% to -8.8% annually, $p < 0.001$). Estimated vaccine-induced population immunity to WPV3 poliomyelitis has improved significantly (around +1% annually, $p < 0.05$) in the North West and North East zones and declined in 2 of the other 4 zones.

Table 5.3a The estimated proportions of the population (aged under 5 years old) that are protected against paralysis by WPV1 through direct immunisation by year and zone. Average change per year and statistical significance of this trend also shown.

Zone	Year					Linear trend (average change per year)	P-value for linear trend
	2005	2006	2007	2008	2009*		
North West	0.24	0.32	0.39	0.45	0.55	0.074	<0.001
<i>Sample size</i>	<i>912</i>	<i>858</i>	<i>730</i>	<i>932</i>	<i>548</i>		
North East	0.30	0.40	0.42	0.55	0.62	0.081	<0.001
<i>Sample size</i>	<i>358</i>	<i>334</i>	<i>336</i>	<i>383</i>	<i>340</i>		
North Central	0.45	0.50	0.50	0.61	0.66	0.053	<0.001
<i>Sample size</i>	<i>531</i>	<i>575</i>	<i>525</i>	<i>666</i>	<i>289</i>		
South West	0.52	0.57	0.65	0.58	0.67	0.029	0.01
<i>Sample size</i>	<i>484</i>	<i>438</i>	<i>453</i>	<i>523</i>	<i>269</i>		
South South	0.49	0.55	0.60	0.59	0.61	0.027	<0.001
<i>Sample size</i>	<i>230</i>	<i>279</i>	<i>429</i>	<i>479</i>	<i>237</i>		
South East	0.46	0.49	0.50	0.53	0.54	0.022	<0.001
<i>Sample size</i>	<i>219</i>	<i>228</i>	<i>274</i>	<i>310</i>	<i>154</i>		
All	0.39	0.46	0.51	0.54	0.61	0.050	<0.001
<i>Sample size</i>	<i>2734</i>	<i>2712</i>	<i>2746</i>	<i>3290</i>	<i>1837</i>		

*January – June

Table 5.3b The estimated proportions of the population (aged under 5 years old) that are protected against paralysis by cVDPV2 through direct immunisation by year and zone). Average change per year and statistical significance of this trend also shown.

Zone	Year					Linear trend (average change per year)	P-value for linear trend
	2005	2006	2007	2008	2009*		
North West	0.47	0.48	0.48	0.38	0.26	-0.047	<0.001
<i>Sample size</i>	<i>912</i>	<i>858</i>	<i>730</i>	<i>932</i>	<i>548</i>		
North East	0.58	0.63	0.56	0.48	0.38	-0.055	<0.001
<i>Sample size</i>	<i>358</i>	<i>334</i>	<i>336</i>	<i>383</i>	<i>340</i>		
North Central	0.81	0.80	0.77	0.64	0.50	-0.072	<0.001
<i>Sample size</i>	<i>531</i>	<i>575</i>	<i>525</i>	<i>666</i>	<i>289</i>		
South West	0.86	0.84	0.78	0.62	0.51	-0.088	<0.001
<i>Sample size</i>	<i>484</i>	<i>438</i>	<i>453</i>	<i>523</i>	<i>269</i>		
South South	0.85	0.83	0.70	0.65	0.62	-0.067	<0.001
<i>Sample size</i>	<i>230</i>	<i>279</i>	<i>429</i>	<i>479</i>	<i>237</i>		
South East	0.83	0.78	0.65	0.60	0.50	-0.083	<0.001
<i>Sample size</i>	<i>219</i>	<i>228</i>	<i>274</i>	<i>310</i>	<i>154</i>		
All	0.71	0.71	0.65	0.54	0.44	-0.068	<0.001
<i>Sample size</i>	<i>2734</i>	<i>2712</i>	<i>2746</i>	<i>3290</i>	<i>1837</i>		

*January – June

Table 5.3c The estimated proportions of the population (aged under 5 years old) that are protected against paralysis by WPV3 through direct immunisation by year and zone. Average change per year and statistical significance of this trend also shown.

Zone	Year					Linear trend (average change per year)	P-value for linear trend
	2005	2006	2007	2008	2009*		
North West	0.31	0.32	0.34	0.33	0.33	0.006	0.006
<i>Sample size</i>	<i>912</i>	<i>858</i>	<i>730</i>	<i>932</i>	<i>548</i>		
North East	0.39	0.41	0.37	0.44	0.44	0.013	0.05
<i>Sample size</i>	<i>358</i>	<i>334</i>	<i>336</i>	<i>383</i>	<i>340</i>		
North Central	0.58	0.57	0.54	0.55	0.52	-0.014	<0.001
<i>Sample size</i>	<i>531</i>	<i>575</i>	<i>525</i>	<i>666</i>	<i>289</i>		
South West	0.65	0.63	0.57	0.63	0.61	-0.009	0.34
<i>Sample size</i>	<i>484</i>	<i>438</i>	<i>453</i>	<i>523</i>	<i>269</i>		
South South	0.62	0.60	0.47	0.53	0.54	-0.023	0.14
<i>Sample size</i>	<i>230</i>	<i>279</i>	<i>429</i>	<i>479</i>	<i>237</i>		
South East	0.59	0.54	0.43	0.48	0.48	-0.031	0.03
<i>Sample size</i>	<i>219</i>	<i>228</i>	<i>274</i>	<i>310</i>	<i>154</i>		
All	0.51	0.50	0.45	0.48	0.48	-0.008	0.18
<i>Sample size</i>	<i>2734</i>	<i>2712</i>	<i>2746</i>	<i>3290</i>	<i>1837</i>		

*January – June

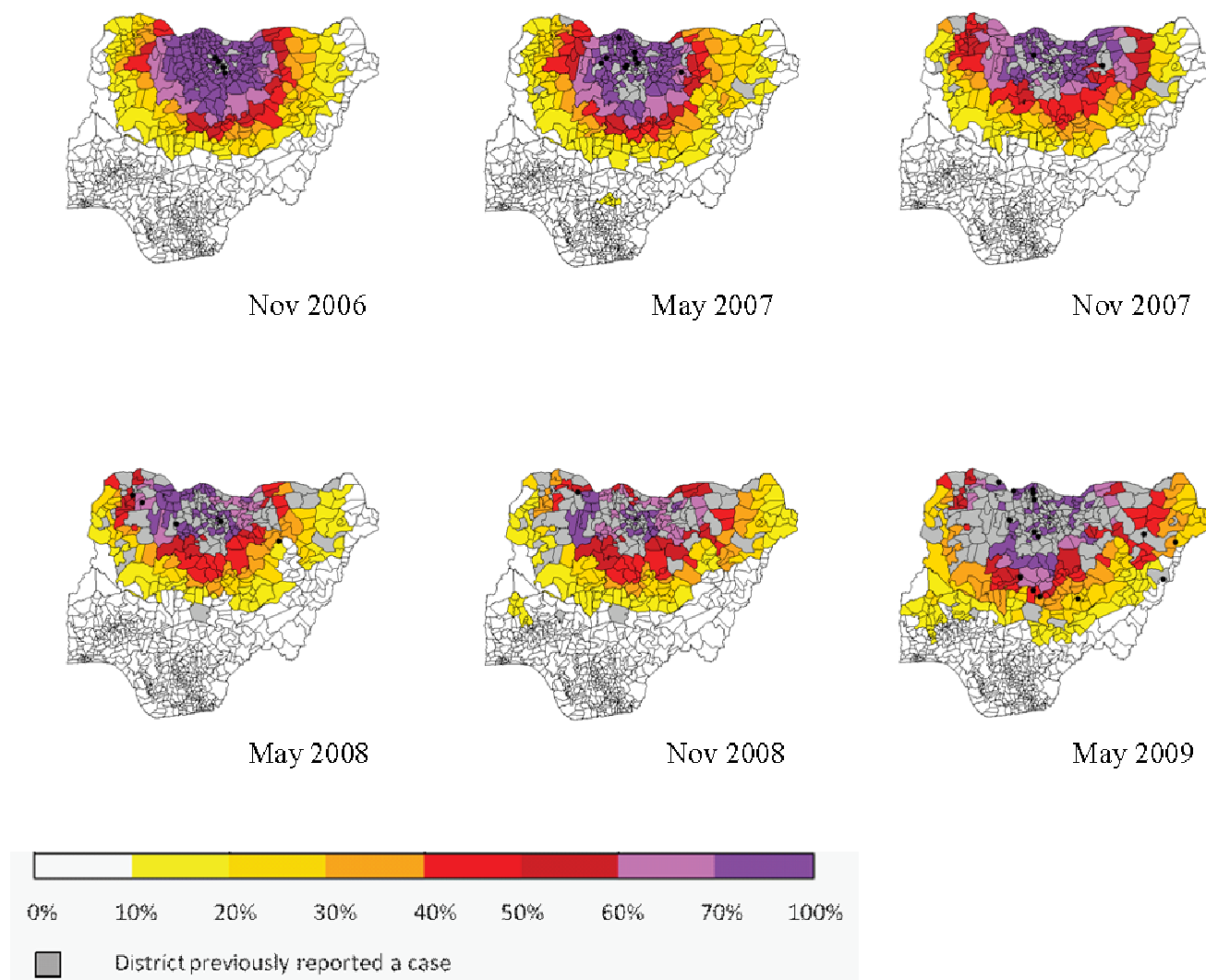
5.3.4 Evolving incidence of cVDPV2 in time and space

The final proportional hazards model for a district reporting its first cVDPV2 case included a function of the distance from other reported cVDPV2 cases, vaccine-induced population immunity (by state) in the previous 6 months, and an interaction term between these two variables (Table 5.4, Figure 5.5, Figure 5.6). This suggests strongly localized risk associated with the well-defined geographic spread of this virus. The interaction term indicates that increases in immunity levels were associated with a greater proportional reduction in the hazard when fewer cases had been reported nearby. Variables based on population numbers or density were excluded from the final model as they were not statistically significant nor confounded any other variables in the model substantially. The final model satisfied the assumption of proportional hazards ($p=0.25$).

Table 5.4 Predictors for a district reporting its first case of cVDPV2

Variable	Relative hazard (95% CI)	z-statistic	P-value
Square of the median distance from cases reported in the previous six months / 1000	0.0022 (-0.0042, 0.0086)	0.69	0.49
Immunity level across the state in the previous six months	-0.103 (-2.34, 2.13)	-0.09	0.93
Interaction between the above two variables	-0.019 (-0.033, -0.006)	-2.85	0.004

Figure 5.5 The estimated hazard of a district reporting its first cVDPV2 case by month from October 2006 – June 2009 based on the fit of the proportional hazards model. Black dots indicate cases of cVDPV2 reported during that month. Only biannual maps shown, for full series of maps, see Figure 5.6.



Relative hazard of a district reporting its first case of cVDPV2 compared to an area which is 0km from all cases reported in the previous 6 months and has 0% population immunity

5.4 Discussion

This study demonstrates that the pathogenicity of this cVDPV2 in Nigeria, and the severity of paralytic disease resulting from infection, are similar to those of WPVs. This study also provides the first estimates of the efficacy of an OPV against paralysis by a cVDPV and demonstrates the greater protective effect of tOPV against this cVDPV2 compared with WPV1 and WPV3. This is consistent with the higher rates of seroconversion to this serotype observed after administration of tOPV in developing countries²⁷. Our results indicate that interrupting the transmission of a cVDPV can require control measures and levels of population immunity similar to those needed to interrupt an outbreak caused by WPV.

Equivalent severity of disease caused by cVDPVs and WPVs is consistent with the common mechanism of disease responsible for poliovirus-associated paralysis⁹². In districts where cVDPV2 and WPV co-circulated, the clinical attack rate for cVDPV2 was only slightly lower than for WPV1 and WPV3, consistent with the lower clinical attack rate for WPV2 compared with WPV1¹⁰. This was observed despite the recent introduction and spread of cVDPV2, and suggests that both the pathogenicity and transmissibility of this virus are similar to WPV. This agrees with a study of serotype 1 cVDPV in Indonesia (45 cases) where limited co-circulation of WPV1 (8 cases) suggested similar attack rates⁹³.

While the 11 other previously recognised cVDPV outbreaks were terminated with between 1 and 4 immunization campaigns with either tOPV or serotype-specific mOPV³⁸, the outbreak in Nigeria persisted despite 6 large-scale and several smaller-scale tOPV campaigns. Given the relatively high estimated efficacy of tOPV against cVDPV2, its persistence can be attributed to inadequate tOPV coverage achieved during SIAs. Furthermore, the 6 large campaigns were spread over 4 years and were interspersed with several mOPV rounds necessary to control WPVs. By 2009, persistent coverage gaps during SIAs, combined with low levels of routine immunization resulted in a further widening of the

immunity gap and the upsurge in cVDPV2 cases observed in the 1st half of 2009. The marked drop in cVDPV2 cases in the 2nd half of 2009 (10 cases in July-December vs. 138 cases in January-June) following just 2 large-scale, moderate quality tOPV rounds in May and August, is consistent with the high efficacy of this vaccine against cVDPV2 and bodes well for the interruption of this virus.

We have previously discussed the limitations associated with estimating vaccine efficacy and population immunity from reported doses of OPV received by AFP cases^{7, 48, 75}. Although these limitations may affect the accuracy of our estimates, the findings of a greater protective efficacy of tOPV against cVDPV2 compared with WPV1 or WPV3, and the better performance of the mOPVs, are robust to these limitations. Furthermore, our estimates of population immunity are predictive of the epidemiology of this cVDPV2. Since cases are less likely to have received the more efficacious vaccine against the polio serotype with which they were infected, a case-control approach based on the inferred type of vaccine received can underestimate the efficacy of mOPVs. Interestingly, there was a borderline significant protective efficacy of mOPV3 against paralysis by WPV1. While this may be a genuine effect due to cross-immunity as has been shown in the past⁹⁴, this result may also indicate erroneous inference of mOPV1 or tOPV doses by the algorithm that we have used. Despite these potential biases, the estimated efficacy of mOPVs was high compared to tOPV and provides a conservative estimate of the efficacy of these vaccines compared with tOPV. Whether the borderline significant efficacy of mOPV3 indicates cross-immunity or an error, our population immunity levels may be incorrect, as a result. If there is cross-immunity then our immunity levels against mOPV3 will be under-estimates. If there has been some erroneous inference then the error could be in either direction. Although we do not account for the number of asymptomatic infections associated with polioviruses in our hazard analysis, we make the reasonable assumption that the number of AFP cases with poliovirus in their stool is proportional to the number of infections with this virus. Additionally, although 4 lineages of ‘circulating’ (>1 AFP case) serotype 2 VDPV emerged during the study period, the current outbreak is largely the result of a single

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lineage (>90% of cases) (Nigeria Expert Review Committee 2009, unpublished data). Our estimates of vaccine-induced immunity do not account for secondary spread of OPV, maternal antibodies early in life, or asymptomatic infection with circulating polioviruses. This may affect estimates of the attack rates among susceptible children. However, estimates of the attack rates among all children agree with our finding of only slightly lower rates for cVDPV2 (Table 5.2).

These findings have important short- and long-term implications for the GPEI. First, this analysis supports the strengthening of current recommendations for responding to cVDPVs to align them with those for WPVs. Secondly, while state-level SIAs have been sufficient to interrupt cVDPVs in most settings, the model shown in Figure 5.5 demonstrates that additional large-scale tOPV rounds, such as those conducted in May and August 2009, are required across northern Nigeria. Finally, the GPEI's plans for managing post-WPV risks must encompass scenarios under which transmissible and pathogenic cVDPVs could emerge, particularly immediately following the cessation of OPV use globally⁹⁵. For example, these data inform the development of long-term polio immunization policy in countries which are currently using OPV but may consider either time-limited or indefinite use of IPV following OPV cessation due in part to the risks posed by cVDPVs.

The eradication of WPV2 globally in 1999 was a remarkable achievement. However, this study demonstrates the fragility of that achievement due to the pathogenicity and severity of disease that can be associated with a serotype 2 cVDPV. Fortunately, tOPV is highly protective against this cVDPV and modest gains in coverage now appear to be facilitating its rapid interruption. The steep decline in WPV cases in northern Nigeria during the second half of 2009 and concomitant declines in cVDPV2 that have followed improvements in SIA coverage⁹⁶ together with the potential role of recently licensed bOPV⁹⁷ improves the prospects for the eradication of these polioviruses in Nigeria.

6. Risks factors for high incidence of paralysis by wild poliovirus serotype 1 in Nigeria

6.1 Summary

Background Nigeria is the only country in Africa that has never interrupted transmission of WPV1.

During 2008/9, cases of WPV1 were reported in 12 other African countries and traced back to polioviruses originating in northern Nigeria. Understanding the risk factors for WPV1 cases in Nigeria will help inform strategies for elimination of polio in Nigeria and thus reduce spread to the rest of the continent.

Methods A negative binomial regression model was developed using data on 3,315 WPV1 cases and 30,002 non-polio AFP cases in the national surveillance database to assess the associations between potential risk factors and monthly incidence of WPV1 by state in Nigeria.

Results While adjusting for other potential risk factors, both low population immunity levels (through direct immunization with OPV in Supplementary Immunisation Activities (SIAs)) and a low proportion of the population receiving 3 routine doses of tOPV by 52 weeks were highly statistically significantly associated with greater incidence of WPV1 cases. There were also significant positive associations between WPV1 incidence and the number of cases reported in the state and in bordering states in the previous six months. There were no significant associations found between population density and WPV1 incidence.

Conclusions Good quality vaccination coverage in both SIAs and routine immunization services are fundamental to WPV1 elimination within Nigeria. This, in turn, could result in the end of wild poliovirus serotype 1 in Africa.

6.2 Introduction

Since the World Health Assembly resolved to eradicate polio in 1988⁴⁵, the disease has been eliminated from all but four countries worldwide: India, Nigeria, Afghanistan and Pakistan. The continuing persistence of WPV in Nigeria is concerning and damaging to the GPEI for two main reasons. Firstly, the actual incidence in the context of global cases is high: for 5 of the last 6 years (2003-2008), Nigeria has seen the greatest burden of paralysis due to WPV in any country worldwide. Secondly, a large number of previously polio-free countries have been re-infected throughout parts of Africa and further afield in recent years. Between 2002 and 2006, polioviruses originating in Nigeria were found to have paralysed children in 19 other countries including Saudi Arabia, Yemen and Indonesia⁵⁸. During 2008/9, further exportations from Nigeria resulted in paralysis cases occurring in 12 countries in Africa (Benin, Burkina Faso, Chad, Ivory Coast, Ghana, Mali, Niger, Togo, Ethiopia, Kenya, Sudan and Uganda)⁹⁸ (data as of April 2009). Notably, all of these countries were infected with WPV1. Elimination of WPV1 within Nigeria will not only be a remarkable step forward for the eradication initiative in terms of progress in that country but will have the added benefit of considerably reducing the risk of WPV1 spread to other countries in Africa.

The continuing persistence of polio in Nigeria has been attributed to poor vaccine coverage with OPV particularly in Northern states^{61, 75} (see Chapter 4). Poor coverage could have occurred in either SIAs or in routine immunization activities or both. While we have shown an association between poor vaccine coverage and higher incidence of WPV1 in Chapter 4, it is important to confirm that this association exists whilst adjusting for other potentially confounding factors. We will investigate the impact of both coverage in SIAs and routine services on the incidence of WPV1. Transmission of polio, and indeed that of other infectious diseases has also been attributed to other factors which may also play a part in the continuing transmission in Nigeria. In India and a number of other countries, population density has been

positively associated with increased polio transmission^{4, 7} as well as a number of other infectious diseases⁹⁹.

Here, we investigate risk factors for reporting cases of paralysis due to WPV1 in Nigeria and compare them with those found in India⁷. We will also develop a model that can be used to identify the states in Nigeria that are at greatest risk of reporting high numbers of WPV1 cases in the near future.

6.3 Results

During the study period there were 3,315 WPV1 cases largely in the northern states of Nigeria (see Appendix Figure A1).

A final negative binomial regression model was developed to model the incidence of these cases which included the variables as described in Table 6.1.

Table 6.1 Parameter estimates for the final negative binomial regression model selected to estimate number of monthly reported WPV1 cases by state

Variable	Parameter estimate (95% confidence interval (CI))	P-value
Natural log of population immunity level	-0.39 (-0.55, -0.23)	<0.001
Natural log of population size	0.82 (0.55, 1.10)	<0.001
Natural log of routine vaccination rate (NICS 2006)	-0.34 (-0.56, -0.11)	0.003
Natural log of number of WPV1 cases reported in bordering Nigerian states/population	0.52 (0.39, 0.66)	<0.001
Natural log of number of WPV1 cases reported in previous six months in the state in question/population (B)	1.08 (0.90, 1.25)	<0.001
Interaction between A and B	-0.17 (-0.21, -0.13)	<0.001

6.3.1 Risk factors for reporting WPV1 cases

After adjusting for other explanatory variables, population immunity levels were found to be strongly linearly negatively associated with the reported number of WPV1 cases ($p < 0.001$). A 25% increase in the immunity level in a state was associated with an 8.3% (95% CI: 5.0% - 11.5%) reduction in number of WPV1 cases reported. Similarly, the proportion of infants immunized with the 3 doses of tOPV recommended for routine immunization was linearly negatively associated with the reported number of WPV1 cases ($p = 0.003$). A 25% increase in the proportion of children immunized with the 3 routine doses of tOPV in a state was associated with a 7.3% (95% CI: 2.5% - 11.8%) reduction in number of WPV1 cases reported. The population in that state was positively linearly associated with the number of WPV1 cases reported ($p < 0.001$) and the parameter estimate was not significantly different from 1 (parameter estimate = 0.82 (95% CI: 0.55 - 1.10)). An increase in the population in a state of 2.38% (i.e. the current annual population growth⁷⁷) was associated with a 1.96% (95% CI: 1.30% - 2.62%) increase in WPV1 cases. The number of WPV1 cases in bordering Nigerian states and in the state in question in the previous 6 months were positively associated with increases in WPV1 cases and there was also a significant interaction between these two variables (Table 6.2). There were no significant associations between number of WPV1 cases reported and the other variables investigated.

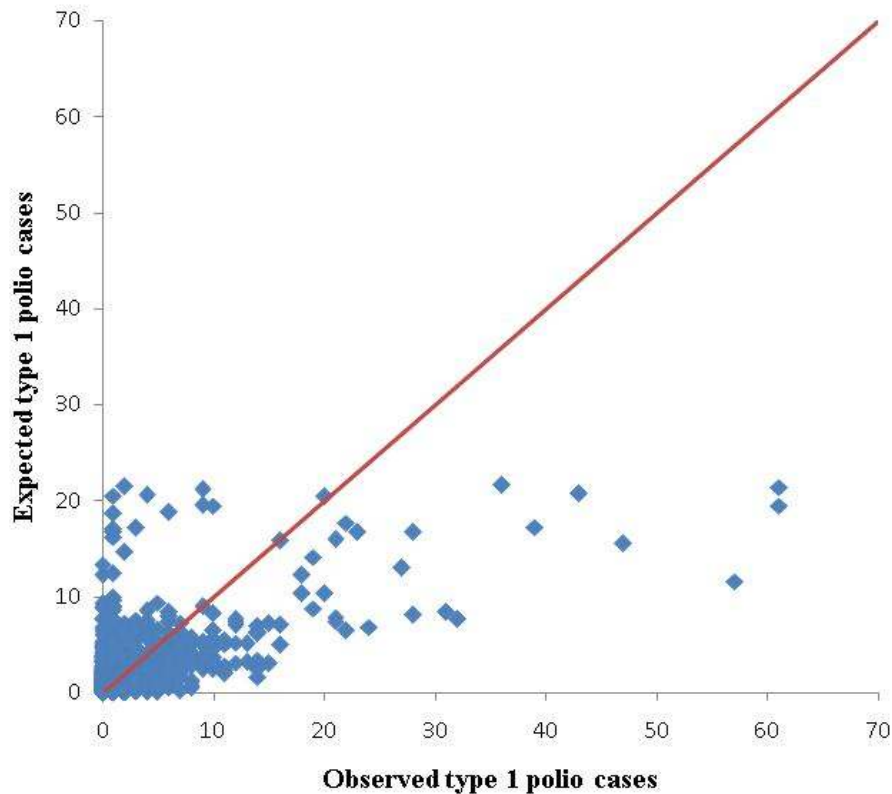
Table 6.2 The percentage increase in the incidence of WPV1 cases associated with increases in the number of WPV1 cases in the same state and in bordering Nigerian states in the previous six months assuming other variables in the model remain constant

		% increase in WPV1 cases in the previous six months in bordering Nigerian states					
		0%	20%	40%	60%	80%	100%
% increase in WPV1 cases in the previous six months in the same state	0%	0%	10%	19%	28%	36%	44%
	20%	22%	33%	44%	53%	62%	71%
	40%	44%	56%	68%	79%	89%	98%
	60%	66%	80%	92%	104%	115%	125%
	80%	88%	103%	117%	130%	141%	152%
	100%	111%	127%	141%	155%	167%	179%

6.3.2 Goodness of fit of the regression model for reported WPV1 cases

Examining the data graphically (Figure 6.1), the regression model appears to correctly identify those states that report low numbers of WPV1 cases (less than 10) during one month. However, for the small number of states that have reported large numbers of WPV1 cases (more than 30) in one month, the model consistently underestimates the number of cases reported.

Figure 6.1 Correlation between the levels of observed WPV1 cases by month and state and those expected based on the final regression model. The straight red line shows the line along which the blue dots would be plotted if there was perfect correlation.



Seventy-eight percent of the state-months reported 0 WPV1 cases while a further 21% reported below 11 and greater than 0. Only 1% reported greater than 10 and were thus classed as high risk. Table 6.3 shows the frequency of observations which fall into each of the three groups and how their expected values based on the final negative binomial regression model compare.

Table 6.3 Frequency of observations in low, medium and high risk groups and how their model-based expected values compare

		Risk groups by observed value		
		Low	Medium	High
Risk groups by expected value	Low	2511	253	0
	Medium	248	459	29
	High	5	24	20

The model that we have developed would put 84% $((2511+459+20)/3549)$ of state-months into the correct risk group. A further 8% $((253+29)/3549)$ would have been categorized as one risk group below that in which they actually fell. No state-months were categorized as low risk when they were actually high risk. The model would have put 8% in a risk group one above that in which they actually fell. Five state-months (0.1%) would be incorrectly classed as high risk when in fact 0 WPV1 cases were reported.

6.4 Discussion

This study has confirmed the importance of immunization with OPV in reducing incidence of WPV1 in Nigeria. Even after adjusting for other potential risk factors, immunization in SIAs and in routine immunization services are both highly significantly associated with the incidence of WPV1 – a 25% increase in either the population immunity level or the proportion of children immunized with 3 doses of tOPV through routine immunization was associated with around an 8% decrease in number of WPV1 cases.

In contrast with a similar study in India⁷, this study found no statistically significant association between incidence of WPV1 in Nigeria and population density. In fact, the northern states of Nigeria are generally very sparsely populated relative to those states in the south which have experienced far fewer WPV1

cases. Even adjusting for the highly heterogeneous immunization rates, population density does not appear to be a contributory factor to WPV1 incidence in Nigeria.

As discussed in previous chapters, our estimates of population immunity do not account for secondary transmission of vaccine virus, maternal antibodies in the first few months of life or natural immunity from exposure to circulating WPV1. Despite this, the immunity levels are comparable across states although the time variation may have introduced a slight bias. It is likely that natural immunity to WPV1 will be higher following a localized outbreak and with the reduction in susceptible children there will be fewer WPV1 cases. If additional SIAs were carried out as a result of the outbreak then there is a possibility that our population immunity estimates would be higher just at the point when WPV1 cases would naturally have decreased. This scenario may have accentuated our result although it is unlikely to account for such a highly significant association. The proportion of children reporting zero doses was not statistically significant when adjusting for population immunity levels and rates of routine immunization. This confirms that this variable is not sufficient as a means of assessing vaccination coverage and that a population immunity level based on a per-dose efficacy is a more accurate way to measure the levels of immunity in a population in Nigeria.

Our data on WPV1 incidence in bordering states only include those within Nigeria. States on the border of Nigeria are at risk of infection from the countries on which they themselves border. Since data from the countries which border Nigeria were not available, the estimates for those bordering states will be underestimates and this would have caused some error in our analysis. However, while the predicted WPV1 incidence in some of the border states is under estimated by the model this does not account for all of the underestimates shown in Figure 6.1 (Appendix Figure A2). Of the 9 state-months that reported more than 30 cases and whose incidence has been underestimated by the model, 8 are Kano state and 1 is Katsina. All the months are in the first 6 months of the calendar year. Kano is the state which has reported

more polio cases than any other for the majority of the last few years. Our model does underestimate these particularly high values and a more dynamical model may theoretically better capture these outliers more effectively. However, given limitations in the dataset, it would be hard in practice to achieve accurate results with such a model.

As with any statistical model, our final model can only inform us about the potential risk factors for which data were available. One potentially important risk factor which could not be included was a measure of people movement. This may, to some extent explain the differences between incidence in the north and south of Nigeria if we assume that there is more movement between the east and west than north and south. However, the model does give a good indication of which state-months were “low”, “medium” or “high” risk, identifying the risk category correctly in 84% of cases and therefore could be used to identify states that should be targeted with SIAs.

In 2009, the number of WPV1 cases reported dropped from 721 in 2008 to 75 as population immunity levels rose (Figure 5.1). As of 20 April there have been no WPV1 cases in 2010 and vaccine coverage in SIAs has reportedly further improved (pers. comm. R.B.Aylward). This study and, indeed, these recent achievements demonstrate how fundamental immunization is to polio elimination in Nigeria. Maintaining and further improving the immunization rates in both SIAs and through routine immunization are key to eliminating WPV1 in Nigeria and preventing any further future spread to the rest of Africa.

7. Discussion

It is hard to dispute that the achievements of the GPEI have been remarkable: eliminating WPV1 and WPV3 from the vast majority of countries and eradicating WPV2 thereby relegating polio to the status of a virtually “forgotten” disease for much of the world. The global eradication of all WPVs remains tantalizingly close. However, the ultimate goal of eradication of all circulating polioviruses has been complicated by the emergence over the last decade of cVDPVs^{2, 38, 100}. The persistence of WPVs in the last four remaining endemic countries highlights the need for highly accurate surveillance and data collection for poliovirus to enable epidemiological analysis of good quality data. This can help to ascertain the reasons for persistence of disease and potential ways to achieve elimination and eradication.

Every year from 2003 to 2006, Nigeria was responsible for the single largest burden of polio in any one country, with incidence reaching a peak in 2006 of 1122 cases of paralysis from WPV² – 56% of the worldwide cases. The situation in Nigeria clearly needed a more in depth understanding and there has been a lack of scientific studies which could tell us why incidence has been so high and how polio might be eliminated.

Here I summarise my findings in the following paragraphs and then go on to place them in the wider context of polio eradication.

The aims of the initial study (Chapter 4) were: to estimate the efficacy of OPVs in Nigeria against paralysis by WPV; assess how vaccine coverage and resulting immunity to paralysis by WPV1 due to vaccination have changed; and learn more about the association between immunity levels and incidence in Nigeria. I demonstrated in this work that the newly introduced mOPV1 was 4 times as efficacious as tOPV against paralysis due to WPV1 and that vaccine coverage had increased between 2005 and 2007 in

the high incidence areas of the country. The combination of these two improvements resulted in increases in population immunity levels (through direct vaccination) against WPV1 across Nigeria. I also showed that, in contrast with the situation in India⁷, OPV efficacy did not differ across the country but in fact vaccine coverage was substantially lower in the high incidence areas. Population immunity levels in Nigeria were correlated with annual incidence of WPV1 indicating that where immunity levels improved, the incidence of WPV1 declined. Indeed further increases in population immunity levels – through continued use of mOPV1 and by increasing vaccine coverage - are a prerequisite for WPV1 elimination.

In 2005, a case of cVDPV2 was reported in Nigeria. Further cases occurred in the following years at a steady rate until 2009 when incidence of this cVDPV2 rose dramatically. The aim of my work in Chapter 5 was to estimate the efficacy of OPVs against all three circulating serotypes of poliovirus in Nigeria (WPV1, WPV3 and cVDPV2) and immunity levels due to vaccination. While there have been other outbreaks of cVDPV globally², only one (in Indonesia⁹³) occurred alongside one or more WPVs. Despite the fact that the existence of cVDPVs had been known for a number of years^{100, 101}, the size of the outbreak in Nigeria and the concurrent circulation of 2 serotypes of WPV provided the first opportunity to compare the clinical characteristics and attack rates of a cVDPV with those of WPVs whilst transmission was ongoing. The per-dose efficacy of mOPV1 against serotype 1 was reassessed (with additional data) to be just over 3 times that of tOPV (per-dose efficacies 43% and 13% respectively). The per-dose efficacy of mOPV3 was around 2.5 times that of tOPV against serotype 3 (per-dose efficacies 53% and 20% respectively). The per-dose efficacy of tOPV against paralysis by cVDPV2 was 38% - comparable with that of mOPV1 against serotype 1 and substantially higher than the efficacy of tOPV against either serotype 1 or 3. I showed that population immunity levels against serotype 1 from 2005 to 2009 rose across the country, levels against serotype 2 levels in all zones fell and against serotype 3, levels remained reasonably stable. Finally, estimates of clinical attack rates and a comparison of clinical features of WPV1, WPV3 and cVDPV2 showed that this cVDPV2 is equally likely to cause paralysis as a WPV2

and has similar clinical characteristics to WPV1 and WPV3. This work has shown that cVDPVs need to be taken as seriously as WPVs although they can be eliminated by applying the same strategies as would be applied to a WPV.

Nigeria is a major source of polio outbreaks in the rest of Africa. It is therefore a substantial barrier to the certification of polio eradication in the WHO African region. In 2008/9, 12 other African countries were re-infected with serotype 1 polioviruses that originated in Nigeria⁹⁸. In Chapter 6, the aims were to identify predictors of incidence of WPV1 in Nigeria. This would help inform strategies to eliminate WPV1 from Nigeria and in turn reduce the risk of re-infection across Africa. I demonstrated that population immunity levels and routine immunization rates were significantly negatively associated with monthly incidence of WPV1 by state. There were also positive associations between WPV1 incidence and the number of WPV1 reported in the same state and neighbouring ones in the previous six months. In comparison with a similar study in India⁷, there was no evidence of an association between the incidence of WPV1 and population density. This indicates that SIAs should be conducted regularly in areas with low population immunity levels with the appropriate OPV serotype and that SIAs should be of a high quality (i.e. as children as possible should be vaccinated at each SIA).

The implications of these results for Nigeria are very positive. I have clearly demonstrated that polioviruses are persisting in Nigeria due to insufficient vaccine uptake in certain parts of the country. This appears to be in contrast to India where high population density is also playing an important role⁷. Nigeria is also without the issues of war and the disruption that brings that face Afghanistan. Although Nigeria is dealing with three poliovirus serotypes simultaneously, there are OPVs that have relatively good efficacy against these polioviruses: just 3 doses of mOPV1 given to each child would provide over 80% immunity which if achieved on a population level, according to Figure 3.3 could be sufficient to eliminate WPV1. Similarly, cVDPV2 could almost certainly be eradicated if every child received the

three recommended doses of tOPV in routine immunization and a further dose through SIAs – 4 doses of tOPV would provide 85% immunity. While polio in Nigeria has been a topic of discussion amongst the medical and scientific community, there had been no quantitative studies of polio in Nigeria until now. Many suspected that a lack of good quality immunization was the cause of continuing polio transmission in Nigeria - simply not enough children were being vaccinated. However, it was important to demonstrate this more conclusively which is what I have done in this thesis. My work has identified the root of the persistence of polio in this country and that the tools exist to achieve elimination. It has also shown what the GPEI has done to achieve the advances that have been made and where efforts now need to be targeted. Considerable work has been done to persuade local leaders and parents and carers of the importance of polio immunization. The challenge now is to overcome logistical and political issues and get OPV to the remaining pockets of children who are unprotected.

This PhD also has implications for the broader picture of polio eradication. As already mentioned, many other African countries which had previously been declared polio-free have been re-infected with polioviruses from Nigeria. In fact, all re-infections with WPV1 in 2008/9 have originated in Nigeria (data as of April 2009⁹⁸) and therefore elimination of this serotype in Nigeria would almost certainly indicate the end of WPV1 in the WHO African region (unfortunately, some WPV3 cases have been reported in African countries originating in India⁹⁸). One of the biggest challenges facing the GPEI is cVDPVs. The outbreak of cVDPV2 in Nigeria provided a unique opportunity to study a cVDPV outbreak which was substantially larger than any previously observed^{2, 38} and was circulating concurrently with WPV1 and WPV3. There has been considerable debate about the dangers posed by cVDPVs. For the first time and as a result of the work in this PhD we can be confident of the properties of a cVDPV although we cannot be certain that all cVDPVs will behave in exactly this way. Since, as stated above, I have shown that cVDPVs can be equally likely to cause paralysis as WPVs but that tOPV can be reasonably efficacious against paralysis due to a cVDPV and that control measures for cVDPVs should be equivalent to those for

WPVs, this work provides information on how public health authorities should respond to a cVDPV outbreak in any country.

A key aspect of the GPEI's post-eradication strategy is the cessation of OPV use once global certification of eradication has been achieved¹⁰². There has been considerable debate over the risks that this would pose^{42, 52, 95}. Following OPV cessation, an increasing proportion of the human population would be fully susceptible to infection with poliovirus and in the event of an outbreak (for example from a laboratory, research site or a polio vaccine manufacturing facility), the effects could be catastrophic⁵⁴. Some have argued that the possibility of a cVDPV outbreak after OPV cessation is a case against stopping OPV use⁵². However, work by Tebbens et al. has estimated that in the year following OPV cessation, the risk of a cVDPV outbreak somewhere in the world is 65-90% and this drops to 5-15% by the end of the second year and 1-5% by the end of the third year^{95, 102}. To support the case for OPV cessation, it was necessary to understand further the dangers that cVDPVs pose in terms of their likelihood of causing paralysis and what strategies would be needed in the event of an outbreak. Since I have shown in Chapter 5 that cVDPVs can be equally pathogenic as WPVs, continuation of OPV post eradication of WPVs will only result in regular cVDPV outbreaks leading to unnecessary paralysis. In Chapter 5, I have argued that strategies for eliminating cVDPVs should be equivalent to those for WPVs - an immediate high quality response with an mOPV of the appropriate serotype should eradicate the cVDPV. When considered alongside the number of children who would be paralysed as a result of VAPP caused by OPV (approximately 1 in 750,000 of first dose recipients²⁵), the work presented in this thesis supports the view that worldwide simultaneous OPV cessation is the only viable option.

The majority of the data used in this thesis has come from a surveillance database. There are several potential issues with using data of this kind. Firstly, it relies on good quality surveillance in the field in Nigeria and confidence that all AFP cases are being identified. While this is more likely in the case of

surveillance for polio cases than it might be for other diseases (due to the nature of an eradication programme and the need to be certain that there are no further cases for certification of eradication), there is still the possibility that some cases might be missed. These might be the ones in the most rural settings and therefore also the least likely to receive OPV. This could have introduced a bias into our population immunity levels that would have resulted in an over estimate of these levels. Another disadvantage of using data from an already established surveillance system is the lack of flexibility. For example, specific items of data are collected for each AFP case and while there might be further data of interest (such as the number of routine doses of OPV that were given or the religion of the AFP case) it is not straightforward to change such a well established system. There are clear issues of data quality, some of which have already been discussed in chapters 4-6. All of the case investigations are conducted after the case has been identified and therefore are prone to reporting and recall bias. Both of these could have had a substantial impact on the reporting of dose numbers although the double-blinded nature of the interviews will have reduced any differences between cases and controls in this regard. Our inference of mOPV and tOPV doses is likely to have caused some errors in the results and the borderline significant protective efficacy of mOPV3 against serotype 1 indicates that this may have happened. The borderline nature of the p-value may have been due to lack of power. The vaccine efficacy analysis should be repeated on a regular basis to ensure the greatest power available is used. As this is done in the near future, it should become apparent if this result was a quirk due to small numbers (if the effect reduces and the p-value gets larger) or a genuine result (if the effect remains and the p-value becomes smaller). Ideally data on the specific numbers of each type of OPV received would be collected to ensure clarity between true cross-immunity and erroneous results. The high ratio of asymptomatic cases to paralytic cases (as described in the Introduction) is always a potential issue when studying polio. With the AFP surveillance system, only the paralytic polio cases are identified and therefore, all the analysis carried out with these data can only focus on the paralysis cases. For example, population immunity levels are only estimating immunity against paralysis, not infection and only due to direct vaccination. We cannot make any account of

maternal antibodies, immunity through natural infection or from secondary vaccine virus. While our population immunity level estimates are useful for comparing levels in different states at different times, they do not support conclusions about immunity levels to infection. Finally, there may be a sampling bias. As shown in Chapter 2, there are more males reporting AFP than females. While there may be a biological reason behind this, we are unaware of any evidence to support this theory. This result was consistent across polio and non-polio AFP cases indicating a potential reporting issue and appears to be consistent across the whole of Nigeria. In terms of this thesis, this issue should not have affected our results substantially. The case control studies in Chapter 5 matched cases to controls on sex and therefore our vaccine efficacy results should not be affected. Our population immunity levels will be based on a greater proportion of males than are present in the general population. In Chapter 2, we showed that males are significantly more likely to have received more OPV doses than females, therefore, our immunity levels may be slightly over estimated. However, the actual difference in the dose distribution between males and females was negligible and therefore this under estimate is unlikely to be substantial. In terms of the relevance of this sampling bias to the eradication programme, the indication is that there may be female polio cases that are going undetected. More active surveillance of girls aged under 5 years for AFP, particularly in high risk polio areas (i.e. near to recently reported polio cases) would be recommended.

There are several lines of research that could be pursued continuing on from the work in this thesis. Using the AFP database, further work could be done examining the non-polio AFP cases that are excreting vaccine virus. Excretion of vaccine virus following receipt of OPV is an indication that the recipient does not have sufficient mucosal immunity against poliovirus infection and could therefore still play a part in transmission of poliovirus. Work by Grassly et al. in 2009³² showed that in India, children who have received more than 12 doses of mOPV1 (and therefore were generally older than the typical children who become paralysed following polio infection) would still excrete vaccine virus and therefore may be

contributing to transmission although not becoming paralysed by poliovirus. Similar work could be undertaken in Nigeria.

The most fruitful area of future research would require access to data on the nucleic acid sequences of the polioviruses circulating in Nigeria. With such data, it would be possible to examine more closely the geographic origin of each case, the speed at which each virus was spreading and the sources of those lineages that have spread to different countries. It would also be possible to construct phylogenetic trees of the polioviruses circulating in Nigeria and through coalescent approaches, estimate the population size of each virus. In 2009, work by Fraser et al.¹⁰³ used such data to estimate the date of origin of the 2009 pandemic H1N1 virus and estimate R_0 (the basic reproductive number⁹⁹, i.e. the average number of secondary cases that would be expected per single infected case in a completely susceptible population) of the 2009 pandemic H1N1 virus. Similar analyses could be possible with appropriate data on polioviruses.

In terms of research within Nigeria, a seroprevalence survey would generate a very valuable dataset. Blood samples and seropositivity results for a large and unbiased sample of the population would provide information on the ratio of paralytic cases to asymptomatic infections in Nigeria. It would also tell us about the levels of infection in different age groups.

The work presented in this thesis has added substantially to current knowledge on polio, especially within Nigeria. We now know where the problems lie in the interruption of polio transmission in Nigeria and where and why the GPEI has been able to tackle these problems successfully. We also have good evidence that further increases in vaccine coverage especially in high incidence areas, targeting with the appropriate OPV, can achieve elimination of all polioviruses circulating in Nigeria. The insights gleaned about cVDPVs can inform strategies both pre- and post-eradication. While the achievements of the GPEI

thus far are remarkable, they are also fragile as evidenced by events such as the cVDPV2 outbreak in Nigeria and a recent outbreak of WPV1 in Tajikistan¹⁰⁴, the first in the WHO European region since certification of eradication in 2002⁴⁷. The future eradication of polio will depend on the capacity of international organizations and individual nations to respond to this continually evolving threat.

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Appendix 1: Case Investigation Form

CASE INVESTIGATION FORM-ACUTE FLACCID PARALYSIS

[APF-C101]

Federal Ministry of Health, Nigeria

Official Use Only		Epid number: _____		Received _____					
		Ctry	State	LGA	Year onset Case no.				
IDENTIFICATION									
LGA: _____		State: _____		Nearest Health Facility to Village: _____					
Address: _____		Village/Neighborhood: _____		Town/City: _____					
Name(s) of patient: _____		Father/Mother: _____							
Date of Birth: ____/____/____		Age: years _____ months _____		Sex: ☐ M=Male ☐ F=Female					
(if DOB unknown)									
NOTIFICATION/INVESTIGATION									
Notified by: _____		Notified: ____/____/____		Date Case Investigated: ____/____/____					
HOSPITALIZATION									
Admitted to hospital ☐		Date of Admission: ____/____/____							
Medical Record No. _____		Name/address of facility: _____							
CLINICAL HISTORY									
Date Onset: ____/____/____		Fever at onset of paralysis ☐		Paralysis progressed 0-3 days ☐					
Of Paralysis: ____/____/____		Flaccid & sudden paralysis ☐		Asymmetrical ☐					
		1=Y, 2=N		1=Y, 2=N					
				Site of Paralysis					
				<table border="1"> <tr> <td>LA</td> <td>RA</td> </tr> <tr> <td>LL</td> <td>RL</td> </tr> </table>		LA	RA	LL	RL
LA	RA								
LL	RL								
				Y, N					
AFTER INVESTIGATION, WAS THIS A TRUE AFP? ☐									
1= Yes If "No", then the rest of the form does not need to be completed Mark "6" for final Classification									
VACCINATION HISTORY:									
Total polio doses ☐		Exclude birth dose							
99=unknown		OPV Birth ____/____/____ 2nd ____/____/____ 4th ____/____/____							
		Doses 1st ____/____/____ 3rd ____/____/____ if > 4, last OPV dose ____/____/____							
STOOL SPECIMEN COLLECTION									
Date 1st Stool: ____/____/____		Date 2nd Stool: ____/____/____		Date Stool Specimen sent to National Lab: ____/____/____					
STOOL SPECIMEN RESULTS									
Date specimen received by National lab: ____/____/____		Condition of stool: ☐		Primary isolation result: P1 P2 P3 NP Ent					
Date result sent by lab to State: ____/____/____		Date result received by State: ____/____/____		1=Y, 2=N 1=Y, 2=N 1=Y, 2=N 1=Y, 2=N					
Date isolate sent from National lab to Regional Lab: ____/____/____		Date differentiation result sent by Regional Lab: ____/____/____		Date differentiation result Received by State: ____/____/____					
				1=Y, 2=N					
FOLLOW-UP EXAMINATION									
Date follow-up examination: ____/____/____		Residual Paralysis? LA RA LL RL		Findings at Follow up: ☐					
		Y, N		1=Residual paralysis 2=No residual paralysis 3=Lost to follow-up 4=Death before follow-up					
FINAL CLASSIFICATION OF THE CASE: ☐									
1=Confirmed 2=Compatible 3=Discarded 6=Not an AFP									
INVESTIGATOR									
Name: _____		Title: _____							
Unit: _____		Address: _____		Phone: _____					
Where has the child been in seeking help for this problem before presenting here (in sequence of visit)?									
(1) Place: _____ duration: _____ (2) Place _____ duration: _____									

- ☐ Copy 1, White: Send immediately to the Data Manager in Abuja after completion
☐ Copy 2, Pink: Send along with stool specimen to the National Laboratory
☐ Copy 3, Green: Send to the Data Manager in Abuja after completing the sixty days follow up examination. Laboratory result and any additional information
☐ Copy 4, Blue: Remains in the Surveillance Officer's File at state level
☐ Copy 5, Yellow: Remains in the CSNO's file at L.G.A. level

Appendix 2: Supplementary Tables and Figures

Table A1a Annual incidence of paralysis due to WPV1 in Nigeria by state

Zone	State	Year					
		2005	2006	2007	2008	2008*	2009*
North West	Jigawa	54	102	6	38	35	1
	Kaduna	67	35	5	47	33	0
	Kano	118	304	10	264	189	1
	Katsina	41	157	8	73	42	1
	Kebbi	83	13	17	9	3	1
	Sokoto	37	31	12	28	16	3
	Zamfara	57	29	2	82	60	2
NORTH WEST		457	671	60	541	378	9
North East	Adamawa	2	1	2	1	1	0
	Bauchi	46	82	16	43	29	1
	Borno	11	24	20	24	18	2
	Gombe	8	11	3	13	7	0
	Taraba	3	1	2	2	2	0
	Yobe	15	25	8	20	13	1
NORTH EAST		85	144	51	103	70	4
North Central	Benue	5	0	0	2	2	5
	FCT	2	2	1	8	4	1
	Kogi	2	0	1	7	0	5
	Kwara	1	1	0	8	2	2
	Nasarawa	8	7	0	5	2	4
	Niger	10	17	3	14	10	5
	Plateau	2	4	0	13	11	1
NORTH CENTRAL		30	31	5	57	31	23
South West	Ekiti	0	0	0	0	0	0
	Lagos	1	0	0	3	0	4
	Ogun	0	0	0	1	0	13
	Ondo	0	0	0	1	0	0
	Osun	0	0	0	2	0	0
	Oyo	0	0	0	11	10	2
SOUTH WEST		1	0	0	18	10	19
South South	Akwa Ibom	0	0	0	0	0	0
	Bayelsa	0	0	0	0	0	4
	Cross River	0	0	0	0	0	0
	Delta	0	0	0	0	0	6
	Edo	0	0	0	0	0	1
	Rivers	0	0	0	0	0	0
SOUTH SOUTH		0	0	0	0	0	11
South East	Abia	0	0	0	0	0	0
	Anambra	0	0	0	0	0	0
	Ebonyi	0	0	0	0	0	1
	Enugu	0	0	0	2	1	0
	Imo	0	0	0	0	0	0
SOUTH EAST		0	0	0	2	1	1
Whole country		573	846	116	721	490	67

* Numbers to end of June of that year, hence the column 2008* is a subset of the totals in 2008.

Table A1b Annual incidence of paralysis due to cVDPV2 in Nigeria by state

Zone	State	Year					
		2005	2006	2007	2008	2008*	2009*
North West	Jigawa	0	1	5	5	2	10
	Kaduna	0	1	6	0	0	11
	Kano	0	17	27	4	3	32
	Katsina	0	1	12	4	4	20
	Kebbi	0	0	4	3	1	3
	Sokoto	0	0	5	8	7	4
	Zamfara	0	0	3	14	5	15
NORTH WEST		0	20	62	38	22	95
North East	Adamawa	0	0	0	0	0	1
	Bauchi	1	1	3	8	5	1
	Borno	0	0	2	1	1	8
	Gombe	0	0	0	0	0	0
	Taraba	0	0	0	0	0	0
	Yobe	0	0	1	7	3	4
NORTH EAST		1	1	6	16	9	14
North Central	Benue	0	0	0	0	0	1
	FCT	0	0	0	0	0	0
	Kogi	0	0	0	1	0	0
	Kwara	0	0	0	0	0	1
	Nasarawa	0	0	0	1	1	2
	Niger	0	0	0	6	4	10
	Plateau	0	0	0	0	0	1
NORTH CENTRAL		0	0	0	8	5	15
South West	Ekiti	0	0	0	0	0	0
	Lagos	0	0	0	0	0	0
	Ogun	0	0	0	0	0	0
	Ondo	0	0	0	0	0	0
	Osun	0	0	0	0	0	1
	Oyo	0	0	0	0	0	0
SOUTH WEST		0	0	0	0	0	1
South South	Akwa Ibom	0	0	0	0	0	0
	Bayelsa	0	0	0	0	0	0
	Cross River	0	0	0	0	0	0
	Delta	0	0	0	0	0	0
	Edo	0	0	0	0	0	0
	Rivers	0	0	0	0	0	0
SOUTH SOUTH		0	0	0	0	0	0
South East	Abia	0	0	0	0	0	0
	Anambra	0	0	0	0	0	0
	Ebonyi	0	0	0	0	0	0
	Enugu	0	0	0	0	0	1
	Imo	0	0	0	0	0	0
SOUTH EAST		0	0	0	0	0	1
Whole country		1	21	68	62	36	126

* Numbers to end of June of that year, hence the column 2008* is a subset of the totals in 2008.

Table A1c Annual incidence of paralysis due to WPV3 in Nigeria by state

Zone	State	Year					
		2005	2006	2007	2008	2008*	2009*
North West	Jigawa	29	30	27	2	1	14
	Kaduna	16	18	2	2	2	16
	Kano	109	51	49	7	4	95
	Katsina	38	22	16	9	9	32
	Kebbi	9	59	10	0	0	13
	Sokoto	16	11	12	0	0	12
	Zamfara	8	20	4	3	3	9
NORTH WEST		225	211	120	23	19	191
North East	Adamawa	1	0	0	0	0	1
	Bauchi	12	18	12	12	3	40
	Borno	1	20	5	7	0	16
	Gombe	1	4	0	0	0	9
	Taraba	0	1	1	2	2	0
	Yobe	5	16	10	9	4	14
NORTH EAST		20	59	29	30	9	80
North Central	Benue	0	0	1	0	0	1
	FCT	3	0	0	1	1	0
	Kogi	1	0	0	1	0	0
	Kwara	0	0	1	1	1	0
	Nasarawa	2	0	3	1	1	5
	Niger	2	5	2	1	1	5
	Plateau	0	1	2	8	3	3
NORTH CENTRAL		8	6	9	13	7	14
South West	Ekiti	0	0	0	0	0	0
	Lagos	0	0	4	0	0	0
	Ogun	0	0	0	3	3	0
	Ondo	0	0	0	0	0	0
	Osun	0	0	0	0	0	0
	Oyo	0	0	6	7	6	0
SOUTH WEST		0	0	10	10	9	0
South South	Akwa Ibom	0	0	0	0	0	0
	Bayelsa	0	0	0	0	0	0
	Cross River	0	0	0	0	0	0
	Delta	0	0	0	0	0	0
	Edo	0	0	0	0	0	0
	Rivers	0	0	1	0	0	0
SOUTH SOUTH		0	0	1	0	0	0
South East	Abia	0	0	0	0	0	0
	Anambra	0	0	0	0	0	0
	Ebonyi	0	0	0	0	0	0
	Enugu	0	0	0	0	0	0
	Imo	0	0	0	0	0	0
SOUTH EAST		0	0	0	0	0	0
Whole country		253	276	169	76	44	285

* Numbers to end of June of that year, hence the column 2008* is a subset of the totals in 2008.

Table A2 Supplementary Immunisation Activities (SIA) by date, vaccine type used and location

Date	Vaccine type	Areas included
January 2001	tOPV	All
April 2001	tOPV	All
June 2001	tOPV	All
November 2001	tOPV	All
January 2002	tOPV	All
June 2002	tOPV	Gombe, Kaduna, Katsina
July 2002	tOPV	Bauchi, Borno, FCT, Gombe, Kaduna, Kano, Katsina, Yobe
October 2002	tOPV	All
November 2002	tOPV	All
January 2003	tOPV	Bauchi, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Yobe
March 2003	tOPV	Kebbi, Niger, Sokoto, Zamfara
April 2003	tOPV	Bauchi, Gombe, Jigawa, Kaduna, Kano, Katsina
June 2003	tOPV	Benue, Kogi, Nasarawa
August 2003	tOPV	Kano
September 2003	tOPV	Bauchi, Benue, Borno, Gombe, Jigawa, Kaduna, Katsina, Kebbi, Kogi, Nasarawa, Niger, Sokoto, Yobe, Zamfara
October 2003	tOPV	All
November 2003	tOPV	All
December 2003	tOPV	FCT, Kano, Lagos, Plateau, Taraba
February 2004	tOPV	All except Kano and Zamfara
March 2004	tOPV	All except Kano
September 2004	tOPV	Bauchi, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Zamfara
November 2004	tOPV	All
February 2005	tOPV	All
April 2005	tOPV	All
May 2005	tOPV	All
July 2005	tOPV	Benue, FCT, Kogi, Nasarawa
August 2005	tOPV	Adamawa, Bauchi, Benue, Borno, FCT, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Kogi, Nasarawa, Niger, Sokoto, Taraba, Yobe, Zamfara
September 2005	tOPV	All
November 2005	tOPV	Adamawa, Bauchi, Benue, Borno, FCT, Gombe, Jigawa, Kaduna,

		Kano, Katsina, Kebbi, Kogi, Nasarawa, Niger, Sokoto, Taraba, Yobe, Zamfara
February 2006	tOPV	All except Kebbi, Lagos, Rivers and Taraba
February 2006	mOPV1	Kebbi, Lagos, Rivers, Taraba
March 2006	tOPV	Kebbi, Lagos, Rivers, Taraba
March 2006	mOPV1	All except Kebbi, Lagos, Rivers and Taraba
May 2006	mOPV1	Adamawa, Bauchi, Borno, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Yobe, Zamfara
June 2006	mOPV1	Adamawa, Bauchi, Borno, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Yobe, Zamfara
August 2006	mOPV1	Taraba
September 2006	mOPV1	Adamawa, Bauchi, Borno, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Yobe, Zamfara
September 2006	mOPV1	Taraba
October 2006	mOPV1	Abia, Anambra, Akwa Ibom, Bayelsa, Cross River, Delta, Ebonyi, Edo, Ekiti, Enugu, Imo, Lagos, Ogun, Ondo, Osun, Oyo, Rivers
November 2006	tOPV	Adamawa, Bauchi, Benue, Borno, Gombe, FCT, Jigawa, Kaduna, Kano, Katsina, Kebbi, Kogi, Kwara, Nasarawa, Niger, Plateau, Sokoto, Taraba, Yobe, Zamfara
January 2007	tOPV	Northern states
January 2007	mOPV1	Southern states
March 2007	tOPV	Bauchi, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Sokoto, Yobe, Zamfara
March 2007	mOPV1	147 highest risk LGAs in 17 states (Bauchi, Benue, Borno, FCT, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Kogi, Niger, Plateau, Sokoto, Yobe, Zamfara)
June 2007	mOPV1	Bauchi, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Sokoto, Yobe, Zamfara
July 2007	mOPV3	Bauchi, Borno, FCT, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Sokoto, Yobe, Zamfara
September 2007	tOPV	Bauchi, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Sokoto, Yobe, Zamfara
October 2007	mOPV3	Adamawa, Lagos, Taraba
November 2007	mOPV1	Adamawa, Bauchi, Benue, Borno, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Kwara, Nasarawa, Niger, Sokoto, Taraba, Yobe,

		Zamfara
December 2007	mOPV3	Lagos, Ogun, Oyo
January 2008	mOPV1	All
February 2008	mOPV3	All
March 2008	tOPV	Selected LGAs in Bauchi and Katsina
March 2008	mOPV1	Selected LGAs in Bauchi and Taraba
April 2008	mOPV3	Oyo
April 2008	mOPV1	Bauchi, Borno, Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, Yobe, Zamfara, selected LGAs in Taraba
May 2008	mOPV3	Ogun
May 2008	mOPV1	Bauchi, Borno, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Oyo, Plateau, Yobe, Zamfara, 1 LGA in Borno
May 2008	tOPV	Delta
June 2008	mOPV1	Oyo, Plateau, Sokoto, selected LGAs in Kaduna
June 2008	tOPV	Delta, selected LGAs in Kaduna and Katsina
July 2008	mOPV1	Bauchi, Borno, Enugu, Gombe, Jigawa, Kaduna, Kano, Katsina, Kebbi, Niger, Oyo, Plateau, Sokoto, Yobe, Zamfara
July 2008	tOPV	Abia, Cross River, Delta, Edo, Rivers
August 2008	mOPV1	All northern states, Enugu, Osun, Oyo
August 2008	tOPV	Anambra, Cross River, Ebonyi, Edo
September 2008	tOPV	Akwa Ibom, Cross River, Edo
November 2008	mOPV1	Northern states
December 2008	mOPV1	Southern states

Table A3a Vaccine efficacy estimates by year of onset of paralysis

Year†	WPV1				WPV3		
	Cases	Case-control	mOPV1 efficacy	tOPV	Cases	Case-control	tOPV efficacy
		matches, n (%)	(%) (95% CI)	efficacy (%)		matches, n (%)	(%) (95% CI)
		of cases)		(95% CI)		of cases)	
2001	35	0 (0%)	N/A*	N/A**	21	2 (10%)	N/A**
2002	174	50 (29%)	N/A*	24 (0, 45)	28	9 (32%)	N/A**
2003	190	89 (47%)	N/A*	25 (6, 40)	163	56 (34%)	38 (11, 56)
2004	598	332 (56%)	N/A*	14 (2, 24)	181	109 (60%)	14 (0, 29)
2005	573	275 (48%)	N/A*	18 (7, 27)	253	134 (53%)	15 (0, 31)
2006	846	369 (44%)	80 (53, 92)	9 (0, 20)	276	120 (43%)	13 (0, 30)
2007	116	59 (51%)	34 (0, 77)	30 (0, 58)	170	72 (42%)	15 (0, 36)
Overall	2532	1174 (46%)	67 (39, 82)	16 (10, 21)	1092	502 (46%)	18 (9, 26)

†mid-point between date of onset of paralysis for case and control; *mOPV1 was introduced only in 2006;

**Numbers were too small to estimate efficacy

Table A3b Vaccine efficacy estimates by age at onset of paralysis

	WPV1				WPV3		
Age (years)†	Cases	Case-control matches, n (%) of cases)	mOPV1 efficacy (%) (95% CI)	tOPV efficacy (%) (95% CI)	Cases	Case-control matches, n (%) of cases)	tOPV efficacy (%) (95% CI)
<1	133	45 (34%)	64 (0, 96)	0 (0, 26)	56	16 (29%)	16 (0, 53)
1-2	922	520 (56%)	49 (0, 77)	19 (11, 27)	339	184 (54%)	10 (0, 24)
2-3	755	426 (56%)	75 (22, 92)	13 (3, 22)	348	205 (59%)	20 (8, 31)
3-4	318	129 (41%)	99 (55, 100)	13 (0, 28)	151	70 (46%)	29 (2, 49)
4-5	108	34 (31%)	0 (0, 95)	23 (0, 48)	59	20 (34%)	29 (0, 65)
>5	94	20 (21%)	N/A**	16 (0, 48)	60	7 (12%)	13 (0, 69)
Missing age data*	202				79		
Overall	2532	1174 (46%)	67 (39, 82)	16 (10, 21)	1092	502 (46%)	18 (9, 26)

†mid-point between age at onset of paralysis for case and control; *these cases were excluded from the analysis as cases and controls were matched on age; **Numbers were too small to estimate efficacy

Table A4 Estimated efficacies of the oral polio vaccines against paralysis from WPV1 and WPV3 in Nigeria, 2001- 2007.

Controls matched to cases based on (a) age at onset of paralysis within 6 months, (b) date of onset of paralysis within 5 months, (c) LGA of residence.

Location	WPV1				WPV3		
	Cases	Case-control matches	mOPV1 efficacy (%) (95% CI)	tOPV efficacy (%) (95% CI)	Cases	Case-control matches	tOPV efficacy (%) (95% CI)
North West	1899	861	56 (9, 79)	14 (7, 20)	851	371	15 (5, 25)
North East	437	147	55 (0, 87)	22 (5, 36)	173	67	18 (0, 40)
North Central	154	65	100 (0, 100)	22 (0, 40)	52	16	24 (0, 56)
South	42	21	N/A*	50 (0, 77)	16	8	12 (0, 54)
Overall	2532	1094	61 (26, 79)	16 (10, 22)	1092	462	16 (7, 25)

*There have been no reported type 1 cases in the South since the introduction of mOPV1

Table A5 Estimated efficacy of tOPV and mOPV1 assuming randomly selected cases and controls, or cases only, received 3 doses of tOPV through routine services. The estimated fraction of children receiving 3 doses through routine services was based on the National Immunisation Coverage Survey⁶⁰. See Chapter 3 (Statistical Methods) for further details.

Randomly chosen recipients of routine tOPV	Percentile*	mOPV1 efficacy (%) (95% CI)	tOPV efficacy (%) (95% CI)
Cases and controls	5 th	50 (17, 71)	16.1 (10.3, 21.5)
	50 th	60 (30, 77)	16.8 (11.1, 22.1)
	95 th	70 (46, 83)	17.6 (11.9, 22.9)
Polio cases only	5 th	78 (59, 88)	13.9 (8.1, 19.4)
	50 th	83 (67, 91)	14.5 (8.6, 19.9)
	95 th	88 (75, 94)	14.9 (9.1, 20.4)

*based on rank after the analysis was repeated 100 times

Table A6 The estimated percentages of the population (aged under 5 years old) that are protected against paralysis from WPV1 from direct immunisation by year and state. These figures are presented graphically in Figure 3.2 of the main text and are calculated using (eq. 2) in Chapter 3 (Statistical Methods) assuming vaccine efficacy estimates do not vary across the country.

Zone	State	Year						
		2001	2002	2003	2004	2005	2006	2007
North West	Jigawa	18	27	22	12	13	25	40
	Kaduna	35	30	31	26	24	49	65
	Kano	34	29	24	9	16	28	54
	Katsina	30	31	24	25	22	28	48
	Kebbi	30	35	39	32	40	55	70
	Sokoto	40	27	32	23	26	62	60
	Zamfara	28	37	39	25	13	36	54
NORTH WEST		31	30	29	20	22	38	55
North East	Adamawa	39	43	35	31	40	63	64
	Bauchi	36	28	34	18	20	54	59
	Borno	19	28	26	21	21	43	56
	Gombe	26	34	42	31	43	53	75
	Taraba	24	38	33	39	47	60	58
	Yobe	32	29	32	35	34	45	48
NORTH EAST		30	33	33	27	31	53	60
North Central	Benue	37	37	41	39	54	71	61
	FCT	44	48	52	54	46	69	71
	Kogi	46	41	44	46	46	65	65
	Kwara	44	45	45	43	54	53	59
	Nasarawa	37	44	46	49	53	63	74
	Niger	41	44	44	43	48	62	77
	Plateau	39	36	31	33	41	50	48
NORTH CENTRAL		41	41	42	42	49	62	64
South West	Ekiti	46	55	45	48	50	61	72
	Lagos	45	49	52	59	64	75	85
	Ogun	51	42	53	54	62	82	89
	Ondo	39	48	51	48	48	61	79
	Osun	36	40	48	42	52	58	75
	Oyo	40	46	44	44	49	70	75
SOUTH WEST		43	47	49	51	56	70	81
South South	Akwa Ibom	31	31	45	39	48	68	77
	Bayelsa	27	40	31	35	55	63	78
	Cross River	37	46	50	53	69	69	78
	Delta	43	40	41	42	51	72	73
	Edo	34	52	41	54	59	69	87
	Rivers	45	37	43	42	44	72	81
SOUTH SOUTH		38	40	43	44	53	70	79
South East	Abia	36	48	51	50	57	70	71
	Anambra	46	42	43	48	55	62	68
	Ebonyi	39	44	38	39	40	58	51
	Enugu	49	42	55	40	50	60	67
	Imo	41	40	42	43	44	65	73
SOUTH EAST		43	43	46	44	50	63	67

Table A7 Estimated population immunity levels from direct vaccination against paralysis from WPV1, 2005-7 calculated assuming that all SIA used tOPV. Also shown are the resulting changes in immunity due to coverage changes, the actual changes seen 2005-7 (from data in Table A6) and therefore the fraction of the improvement in vaccine-induced immunity that resulted from the introduction of mOPV1

Zone	State	2005	Assuming no mOPV1		Change from 2005 to 2007 due to coverage changes	Estimated total change from 2005 to 2007	Improvement due to use of mOPV1 (% of total change)
			Year 2006	Year 2007			
North West	Jigawa	13	16	21	8	27	19 (70)
	Kaduna	24	32	41	17	41	24 (59)
	Kano	16	20	34	18	38	20 (53)
	Katsina	22	19	26	4	26	22 (85)
	Kebbi	40	41	42	2	30	28 (93)
	Sokoto	26	37	33	7	34	27 (79)
	Zamfara	13	25	29	16	41	25 (61)
NORTH WEST		22	25	33	11	33	22 (67)
North East	Adamawa	40	42	41	1	24	23 (96)
	Bauchi	20	36	35	15	39	24 (62)
	Borno	21	31	32	11	35	24 (69)
	Gombe	43	43	55	12	32	20 (63)
	Taraba	47	47	40	-7	11	18 (100)
	Yobe	34	30	27	-7	14	21 (100)
NORTH EAST		31	37	37	6	29	23 (79)
North Central	Benue	54	60	52	-2	7	9 (100)
	FCT	46	58	50	4	25	21 (84)
	Kogi	46	54	54	8	19	11 (58)
	Kwara	54	39	47	-7	5	12 (100)
	Nasarawa	53	54	56	3	21	18 (86)
	Niger	48	52	53	5	29	24(83)
	Plateau	41	38	35	-6	7	13 (100)
NORTH CENTRAL		49	51	49	0	15	15 (100)
South West	Ekiti	50	46	50	0	22	22 (100)
	Lagos	64	64	63	-1	21	22 (100)
	Ogun	62	68	69	7	27	20 (74)
	Ondo	48	49	51	3	31	28 (90)
	Osun	52	49	52	0	23	23 (100)
	Oyo	49	55	48	-1	26	27 (100)
SOUTH WEST		56	57	56	0	25	25 (100)
South South	Akwa Ibom	48	53	46	-2	29	31 (100)
	Bayelsa	55	51	49	-6	23	29 (100)
	Cross River	69	57	51	-18	9	27 (100)
	Delta	51	57	47	-4	22	26 (100)
	Edo	59	58	59	0	28	28 (100)
	Rivers	44	51	49	5	37	32 (86)
SOUTH SOUTH		53	55	50	-3	26	29 (100)
South East	Abia	57	56	43	-14	14	28 (100)
	Anambra	55	46	43	-12	13	25 (100)
	Ebonyi	40	41	33	-7	11	18 (100)
	Enugu	50	47	38	-12	17	29 (100)
	Imo	44	51	45	1	29	28 (97)
SOUTH EAST		50	48	41	-9	17	26 (100)

Table A8a Characteristics of all and matched WPV1 cases

	Cases* n (%) (n=2469)	Cases with necessary data** n (%) (n=2171)	Case-control matches n (%) (n=1174)
Age (years)			
<1	128 (5%)	116 (5%)	58 (5%)
1-2	890 (39%)	858 (40%)	536 (46%)
2-3	744 (33%)	721 (33%)	406 (35%)
3-4	308 (14%)	294 (14%)	123 (10%)
4-5	107 (5%)	97 (4%)	31 (3%)
5+	92 (4%)	85 (4%)	20 (2%)
Missing	200 (8%)		
Location			
North West	1861 (75%)	1663 (77%)	925 (79%)
North East	421 (17%)	340 (16%)	158 (13%)
North Central	147 (6%)	134 (6%)	68 (6%)
South	40 (1%)	34 (2%)	23 (2%)
Period			
2001-2003	379 (15%)	320 (15%)	148 (13%)
2004-2007	2090 (86%)	1851 (85%)	1026 (87%)
Exposed to mOPV1	488 (20%)	469 (22%)	237 (20%)

*These case numbers exclude those excreting vaccine virus since these were excluded from our case-control studies

**Necessary data = reported doses and age at onset of paralysis

Table A8b Characteristics of all and matched WPV3 cases

	Cases* n (%) (n=1070)	Cases with necessary data** n (%) (n=946)	Case-control matches n (%) (n=502)
Age (years)			
<1	54 (5%)	53 (6%)	22 (4%)
1-2	327 (33%)	315 (33%)	191 (38%)
2-3	342 (35%)	326 (34%)	198 (39%)
3-4	149 (15%)	143 (15%)	64 (13%)
4-5	59 (6%)	56 (6%)	20 (4%)
5+	60 (6%)	53 (6%)	7 (1%)
Missing	79 (7%)		
Location			
North West	835 (78%)	743 (79%)	402 (80%)
North East	170 (16%)	148 (16%)	74 (15%)
North Central	50 (5%)	41 (4%)	18 (4%)
South	15 (1%)	14 (1%)	8 (1%)
Period			
2001-2003	205 (19%)	180 (19%)	74 (15%)
2004-2007	865 (81%)	766 (81%)	428 (85%)
Exposed to mOPV1	331 (31%)	322 (34%)	171 (35%)

*These case numbers exclude those excreting vaccine virus since these were excluded from our case-control studies

**Necessary data = reported doses and age at onset of paralysis

Appendix Table A9 Site of paralysis of paralytic polio cases by poliovirus type

Left Arm	Right Arm	Left Leg	Right Leg	cVDPV2, n (%)	WPV1, n (%)	WPV3, n (%)
No	No	No	No	0 (0)	11 (<1)	5 (1)
			Yes	81 (29)	626 (27)	126 (12)
		Yes	No	27 (10)	389 (17)	83 (8)
			Yes	39 (14)	604 (26)	127 (12)
	Yes	No	No	3 (1)	26 (1)	4 (<1)
			Yes	9 (3)	92 (4)	21 (2)
		Yes	No	0 (0)	9 (<1)	3 (<1)
			Yes	2 (1)	17 (<1)	4 (<1)
Yes	No	No	No	31 (11)	156 (7)	24 (2)
			Yes	38 (14)	201 (9)	18 (2)
		Yes	No	18 (6)	81 (3)	23 (2)
			Yes	5 (2)	34 (1)	6 (1)
	Yes	No	No	0 (0)	9 (<1)	0 (0)
			Yes	3 (1)	13 (1)	2 (<1)
		Yes	No	0 (0)	4 (<1)	5 (<1)
			Yes	12 (4)	44 (2)	17 (2)
Missing data				10 (4)	7 (<1)	2 (<1)
Total				278	2323	1059

Appendix Table 10 Estimated per dose efficacies of oral polio vaccines against paralysis by the different polioviruses circulating in Nigeria (negative efficacies indicate an increased odds of paralysis due to that serotype per dose of OPV).

Variable	WPV1 (n=2,323)	cVDPV2 (n=278)	WPV3 (n=1,059)
Per dose vaccine efficacy (%; 95% Confidence Interval)			
Number of cases matched to controls (% of total cases)	1739 (75%)	184 (66%)	812 (77%)
mOPV1	34 (19, 46)	-10 (-90, 36)	-14 (-44, 10)
tOPV	14 (9, 18)	39 (11, 58)	21 (13, 28)
mOPV3	39 (-4, 65)	32 (-467, 90)	69 (18, 88)

Appendix Table A11a Estimated per dose efficacy of mOPV1 against paralysis by WPV1 when cases were matched to controls on age at onset of paralysis (within 6 months), date of onset of paralysis (within 6 months), sex and location of residence (district or state, see table)

	Cases matched to controls by district	Cases matched to controls by state
Year†	mOPV1 efficacy (%) (95% CI)	mOPV1 efficacy (%) (95% CI)
2005	N/A*	N/A*
2006	74 (38, 91)	69 (43, 83)
2007	30 (0, 84)	0 (0, 39)
2008	45 (24, 60)	42 (28, 54)
2009	52 (0, 81)	37 (3, 59)
Overall	45 (30, 57)	43 (33, 51)
P-value for interaction	0.39	0.03

†mid-point between date of onset of paralysis for case and control;

*mOPV1 was only introduced in Nigeria in 2006

Appendix Table A11b Estimated per dose efficacy of tOPV and mOPV1 against paralysis by WPV1 when cases are matched to controls on age at onset of paralysis (within 6 months), date of onset of paralysis (within 6 months), sex and location of residence (district or state, see table)

Zone	Cases matched to controls by district		Cases matched to controls by state	
	mOPV1 efficacy (%)	tOPV efficacy (%)	mOPV1 efficacy (%)	tOPV efficacy (%)
	(95% CI)	(95% CI)	(95% CI)	(95% CI)
North West	42 (22, 56)	11 (1, 20)	46 (35, 55)	9 (3, 15)
North East	44 (0, 83)	35 (0, 58)	38 (0,62)	29 (15, 40)
North Central	27 (0, 60)	3 (0, 29)	12 (0, 37)	17 (3, 29)
South	89 (5, 99)	81 (0, 100)	75 (37, 90)	88 (0, 99)
Overall	45 (30, 57)	13 (4, 21)	43 (33, 51)	13 (8, 18)
P-value for interaction	0.14	0.31	0.03	0.002

Appendix Table A12a Estimated per dose efficacy of mOPV1 and tOPV against paralysis from WPV1 assuming randomly selected cases and controls, or cases only, received 3 doses of tOPV through routine services. The estimated fraction of children receiving 3 doses through routine services was based on the National Immunisation Coverage Survey (see Chapter 3 (Statistical Methods)).

Randomly chosen recipients of routine tOPV	Percentile*	mOPV1 efficacy (%) (95% CI)	tOPV efficacy (%) (95% CI)
Cases and controls	5 th	40 (30, 48)	18 (13, 22)
	50 th	35 (25, 43)	16 (12, 20)
	95 th	30 (20, 39)	15 (10, 19)
Cases only	5 th	61 (54, 67)	8 (3, 13)
	50 th	59 (51, 65)	7 (2, 11)
	95 th	56 (48, 62)	6 (1, 11)

*based on rank after the analysis was repeated 100 times

Appendix Table A12b Estimated per dose efficacy of tOPV against paralysis from cVDPV2 assuming randomly selected cases and controls, or controls only, received 3 doses of tOPV through routine services. The estimated fraction of children receiving 3 doses through routine services was based on the National Immunisation Coverage Survey (see Chapter 3 (Statistical Methods)).

Randomly chosen recipients of routine tOPV	Percentile*	tOPV efficacy (%) (95% CI)
Cases and controls	5 th	33 (15, 47)
	50 th	23 (6, 37)
	95 th	14 (0, 29)
Controls only	5 th	55 (37, 68)
	50 th	52 (35, 65)
	95 th	49 (31, 62)

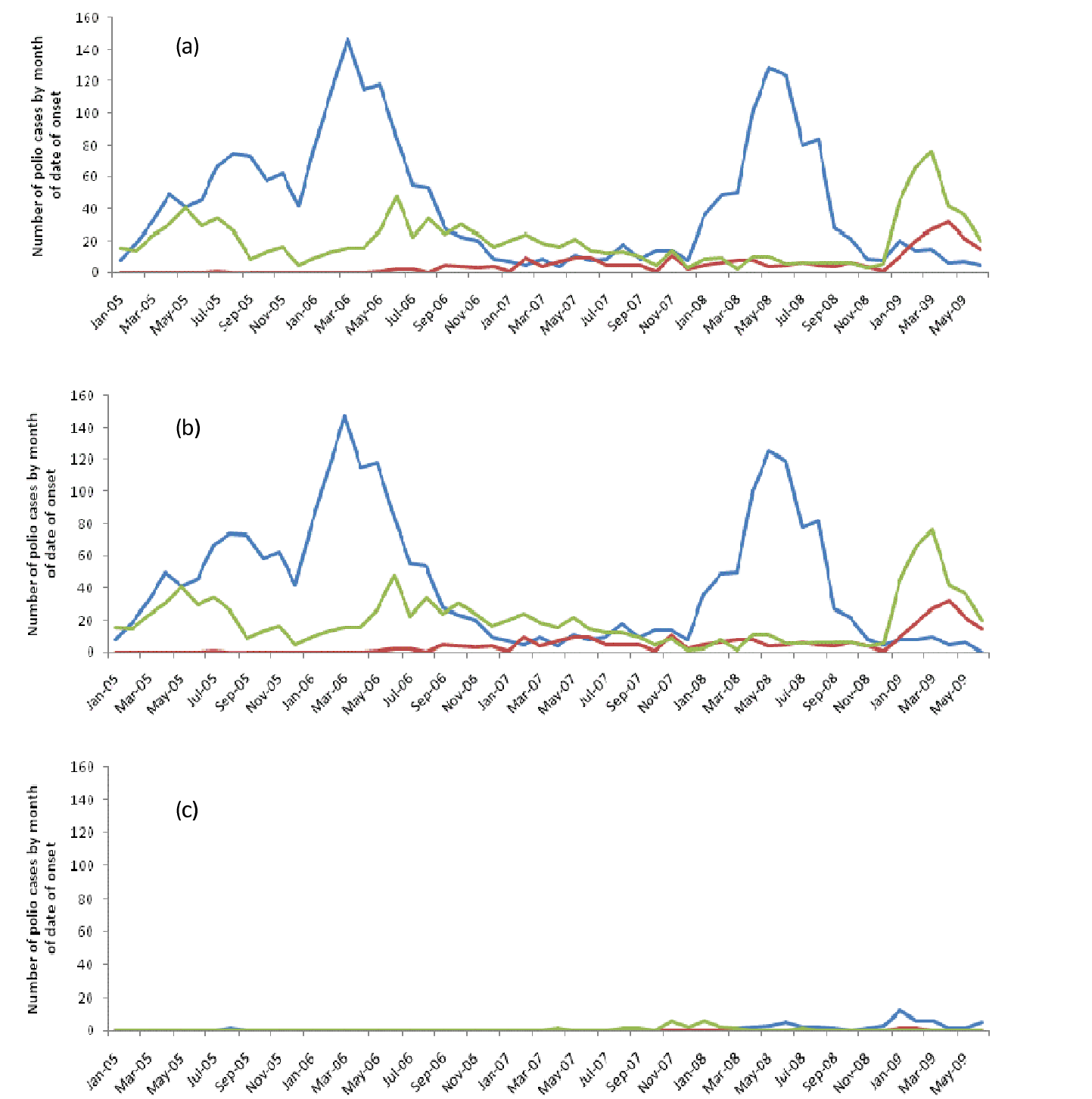
*based on rank after the analysis was repeated 100 times

Appendix Table A12c Estimated per dose efficacy of mOPV3 and tOPV against paralysis from WPV3 assuming randomly selected cases and controls, or cases only, received 3 doses of tOPV through routine services. The estimated fraction of children receiving 3 doses through routine services was based on the NICS (see Chapter 3 (Statistical Methods)).

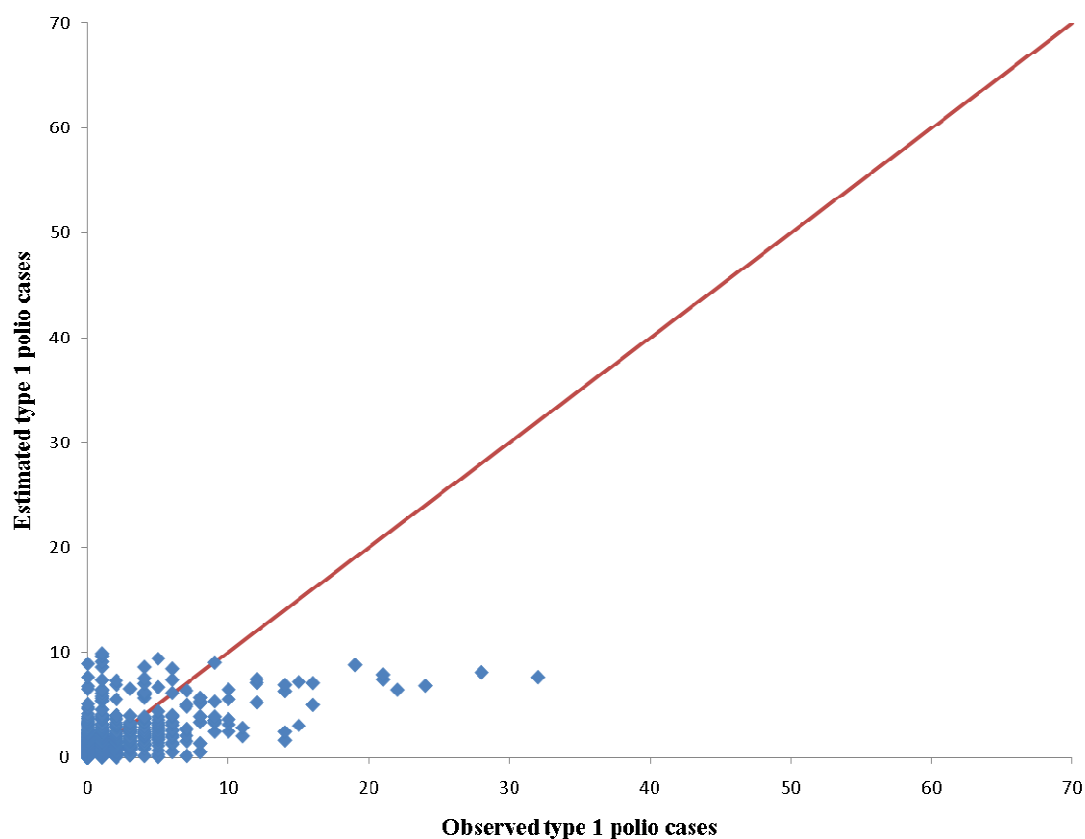
Randomly chosen recipients of routine tOPV	Percentile*	mOPV3 efficacy (%) (95% CI)	tOPV efficacy (%) (95% CI)
Cases and controls	5 th	64 (35, 80)	20 (14, 26)
	50 th	50 (16, 70)	18 (12, 24)
	95 th	40 (1, 64)	16 (10, 22)
Cases only	5 th	84 (67, 92)	12 (5, 18)
	50 th	78 (57, 89)	11 (4, 17)
	95 th	72 (47, 85)	9 (2, 16)

*based on rank after the analysis was repeated 100 times

Figure A1 Number of cases of all three types of polio currently circulating in Nigeria by month of date of onset (January 2001 – June 2009 shown). Blue indicates WPV1, green, WPV3 and red, cVDPV2. Graph (a) data for the whole of Nigeria, (b) northern states only and (c) southern states only.



Appendix Figure A2 Correlation between the levels of observed WPV1 cases by month and state and those expected based on the final regression model. The straight red line shows the line along which the blue dots would be plotted if there was perfect correlation. Only states at the border of Nigeria are shown.



Appendix 3: Published papers by the candidate during the course of this PhD

A. Papers published from this thesis

1. **Jenkins HE**, Aylward RB, Gasasira A, et al. Effectiveness of immunization against paralytic poliomyelitis in Nigeria. *The New England Journal of Medicine* 359:1666-74 (2008)
2. **Jenkins HE**, Aylward RB, Gasasira A, et al. Implications of a circulating vaccine-derived poliovirus in Nigeria. *The New England Journal of Medicine* 362(25):2360-9 (2010)

B. Papers published outside this thesis during this PhD

1. **Jenkins HE**, Woodroffe R, Donnelly CA. The duration of the effects of repeated widespread badger culling on cattle tuberculosis following the cessation of culling. *PLoS One*. 10;5(2):e9090 (2010)
2. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, Griffin J, Baggaley RF, **Jenkins HE**, Lyons EJ, Jombart T, Hinsley WR, Grassly NC, Balloux F, Ghani AC, Ferguson NM, Rambaut A, Pybus OG, Lopez-Gatell H, Alpuche-Aranda CM, Chapela IB, Zavala EP, Guevara DM, Checchi F, Garcia E, Hugonnet S, Roth C; WHO Rapid Pandemic Assessment Collaboration. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science*. 324:1557-1561 (2009)
3. **Jenkins HE**, Woodroffe R, Donnelly CA. The effects of annual widespread badger culls on cattle tuberculosis following the cessation of culling. *International Journal of Infectious Diseases*. 12(5):457-65 (2008)

ORIGINAL ARTICLE

Effectiveness of Immunization against Paralytic Poliomyelitis in Nigeria

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ABSTRACT

BACKGROUND

From the Medical Research Council (MRC) Centre for Outbreak Analysis and Modeling, Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London, London (H.E.J., C.A.D., N.C.G.); the Global Polio Eradication Initiative, World Health Organization (WHO), Geneva (R.B.A.); and WHO–Nigeria (A.G.) and the National Primary Health Care Development Agency (E.A.A., T.K.-A.)—both in Abuja, Nigeria. Address reprint requests to Ms. Jenkins at MRC Centre for Outbreak Analysis and Modeling, Department of Infectious Disease Epidemiology, Faculty of Medicine, St. Mary's Campus, Imperial College London, Norfolk Pl., London W2 1PG, United Kingdom, or at h.jenkins@imperial.ac.uk.

The number of cases of paralytic poliomyelitis has declined in Nigeria since the introduction of newly licensed monovalent oral poliovirus vaccines and new techniques of vaccine delivery. Understanding the relative contribution of these vaccines and the improved coverage to the decline in incident cases is essential for future planning.

METHODS

We estimated the field efficacies of monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine, using the reported number of doses received by people with poliomyelitis and by matched controls as identified in Nigeria's national surveillance database, in which 27,379 cases of acute flaccid paralysis were recorded between 2001 and 2007. Our estimates of vaccine coverage and vaccine-induced immunity were based on the number of doses received by children listed in the database who had paralysis that was not caused by poliovirus.

RESULTS

The estimated efficacies per dose of monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine against type 1 paralytic poliomyelitis were 67% (95% confidence interval [CI], 39 to 82) and 16% (95% CI, 10 to 21), respectively, and the estimated efficacy per dose of trivalent oral poliovirus vaccine against type 3 paralytic poliomyelitis was 18% (95% CI, 9 to 26). In the northwestern region of Nigeria, which reported the majority of cases during the study period, coverage with at least one dose of vaccine increased from 59 to 78%. Between 2005 and 2007, vaccine-induced immunity levels among children under the age of 5 years more than doubled, to 56%.

CONCLUSIONS

The higher efficacy of monovalent type 1 oral poliovirus vaccine (four times as effective as trivalent oral poliovirus vaccine) and the moderate gains in coverage dramatically increased vaccine-induced immunity against serotype 1 in northern Nigeria. Further increases in coverage in Nigerian states with infected populations are required to achieve the levels of vaccine-induced immunity associated with the sustained elimination achieved in other parts of the country.

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BY 2003, THE GLOBAL POLIO ERADICATION Initiative had interrupted the transmission of indigenous wild polioviruses in all but six countries worldwide, including Nigeria.¹ In that year, however, the temporary suspension of all poliovirus immunization in one Nigerian state contributed to a national epidemic of poliomyelitis and the reinfection of at least 20 previously wild-type poliovirus-free countries.² Although poliovirus immunization was reinstituted within 12 months, vaccine coverage remained low across northern Nigeria, and case numbers continued to increase.

In early 2006, Nigerian health authorities adopted new tools and tactics to accelerate poliovirus eradication. To respond to lingering community concerns, Nigeria's national immunization days have been replaced by "immunization-plus days," during which a range of childhood vaccinations and other health interventions are offered along with the oral poliovirus vaccine. To enhance the effectiveness of immunization contacts against types 1 and 3 paralytic poliomyelitis, trivalent oral poliovirus vaccine was replaced with monovalent oral poliovirus vaccine on a number of immunization-plus days from February 2006 to the present (type 1) and from July 2007 to the present (type 3).³⁻⁵ In 2007, type 1 poliomyelitis cases in Nigeria fell by 86% as compared with 2006, contributing to an overall decline in poliomyelitis cases of 75% (see Tables 1A and 1B in the Supplementary Appendix, available with the full text of this article at www.nejm.org). International exportations of poliovirus also plummeted, suggesting a vast improvement in the prospects for global eradication.

Although both the use of monovalent type 1 oral poliovirus vaccine and the implementation of immunization-plus days are generally credited with the progress in 2007, a critical evaluation of the interventions used is essential in assessing the feasibility of completely eliminating wild-type poliovirus in Nigeria and, ultimately, the world. An estimate of the efficacy of the poliovirus vaccines is especially important in the context of the recent resurgence of cases of type 1 poliomyelitis in 2008 in northern Nigeria. We investigated the protective efficacies of monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine in Nigeria, the changes in vaccine coverage after the introduction of immunization-plus days, the resulting vaccine-induced population immunity over time, and the levels of immunity as-

sociated with local interruption of wild poliovirus in this setting. We discuss the implications of these results for the elimination of wild-type poliovirus from Nigeria.

METHODS

DATA COLLECTION

We identified cases of poliomyelitis in the national database maintained by the government of Nigeria, which since December 1996 has recorded all cases of acute flaccid paralysis detected and reported among children under the age of 15 years.⁶ Acute flaccid paralysis is characterized by the rapid onset of weakness and progression to maximum severity within several days to weeks, with the absence of spasticity or other signs of disordered motor tracts in the central nervous system, such as hyperreflexia, clonus, or extensor plantar responses.⁷ Since 2001, the quality of national surveillance for cases of acute flaccid paralysis not caused by poliovirus has exceeded the international performance standard of at least 1 case reported annually per 100,000 children.⁸ Consequently, the cases of acute flaccid paralysis examined had reported dates of onset of paralysis between January 1, 2001, and December 31, 2007. All such cases are subject to standard clinical, epidemiologic, and laboratory investigations, including the collection of two stool samples, at least 24 hours apart, to test for wild and vaccine poliovirus. The number of doses of oral poliovirus vaccine received by each child with acute flaccid paralysis (as reported by a parent or guardian) was recorded during interviews before laboratory testing of the stool samples to ensure blinding of both parents (or other caregivers) and investigators.

Other data collected in the initial interviews included date of birth, date of onset of paralysis, and location of residence (administratively, Nigeria is divided into 6 zones, 37 states, and 774 local government areas). A total of 27,379 cases of acute flaccid paralysis were in the data set. The following cases were excluded from the study: those in which there was residual paralysis compatible with poliomyelitis and for which two adequate stool specimens were not available (3.0%), those in which vaccine virus was isolated from stool (5.4%), and those in which the number of vaccine doses received or the age at the onset of paralysis were not reported (9.3% and 3.9%, respectively). Consequently, a total of 21,815 cases of acute flaccid

paralysis (79.7%) were included in the study (see Fig. 1 in the Supplementary Appendix).

A case of type 1 or type 3 poliomyelitis was defined as any case of acute flaccid paralysis with virologic confirmation of type 1 or type 3 wild poliovirus, respectively, from at least one stool sample. The sensitivity of testing for poliovirus in two stool samples was estimated with the use of published methods⁹ (see the Methods section in the Supplementary Appendix). In this study, cases of acute flaccid paralysis not caused by poliovirus were defined as those in which neither wild nor vaccine-related poliovirus was isolated. Acute flaccid paralysis not caused by poliovirus may result from a range of other conditions, including the Guillain-Barré syndrome and infection with other enteroviruses.⁷ Routine immunization coverage in Nigeria is low; nationwide in 2006, just 37% of children had received three doses of trivalent oral poliovirus vaccine by 1 year of age. Routine coverage is extremely heterogeneous nationwide and particularly low in northern states.¹⁰ Most poliovirus vaccine doses are received through supplementary immunization activities, which were introduced in 1996. We therefore assumed that children received all doses of oral poliovirus vaccine through supplementary immunization activities rather than through routine immunization.

The number of these activities during which a child with acute flaccid paralysis was exposed to each vaccine type was calculated on the basis of the child's date of birth, age at onset of paralysis, and state of residence (see Table 2 in the Supplementary Appendix). The fraction of the rounds in which each vaccine type was used was multiplied by the number of reported doses received to yield an expected number of doses of each type received.¹¹ The sensitivity of the results to the assumption that all doses were received by means of supplementary immunization activities was assessed by assuming that the first three doses received by randomly chosen children in the analysis were trivalent oral poliovirus vaccine provided during routine services and that the remaining doses were provided during supplementary immunization activities and consisted of monovalent type 1 oral poliovirus vaccine, trivalent oral poliovirus vaccine, and monovalent type 3, as described above. The fraction of children assumed to have received routine trivalent oral poliovirus vaccine doses in this sensitivity analysis was based

on the reported routine immunization coverage in each zone¹⁰; the random analysis was repeated 100 times (see the Methods section in the Supplementary Appendix). The potency of each monovalent vaccine is equivalent to the individual components of the trivalent vaccine. Thus, a dose of monovalent type 1 oral poliovirus vaccine contains 10^6 median cell-culture infectious doses (CCID₅₀), and a dose of monovalent type 3 oral poliovirus vaccine contains $10^{5.8}$ CCID₅₀, as found in the trivalent formulation.

Surveillance includes investigation of all potential cases of vaccine-associated paralytic poliomyelitis. Among children who have received the first dose of vaccine, the incidence of vaccine-associated paralytic poliomyelitis is 1 case per 1 million children, and the incidence is much lower after subsequent doses.^{12,13} The Expert Review Committee in Nigeria examines all potential cases of vaccine-associated paralytic poliomyelitis and has reported no increase in the incidence since the introduction of monovalent type 1 oral poliovirus vaccine. Analysis of the National Surveillance database, which tracks the use of standard vaccines licensed by the National Regulatory Authority of the Government of Nigeria for use in Nigeria, without potential for individual case identification, does not require institutional ethics approval. All authors vouch for the completeness and accuracy of the data reported.

STATISTICAL ANALYSIS

Two matched case-control studies (one for type 1 poliovirus, and one for type 3) were used to estimate the efficacies of the vaccines against paralytic poliomyelitis (type 2 wild poliovirus was eradicated globally in 1999). Children with poliomyelitis were matched in a 1:1 ratio with controls randomly selected from the database of children with acute flaccid paralysis not caused by poliovirus; matching was based on the age at onset of paralysis (within 6 months), the date of onset of paralysis (within 6 months), and region (same local government area). These matching criteria were chosen to maximize the number of case-control pairs while minimizing the biases that might be introduced by a failure to control for differences in exposure between cases and controls. Children with acute flaccid paralysis that was not caused by poliovirus were considered appropriate controls because all cases of acute flaccid paralysis were investigated before confirmation

of poliovirus status, ensuring the blinding of investigators and parents (or guardian) during dose-reporting interviews. Conditional logistic-regression analysis of the 1:1 matched data was used to relate the log-odds of disease to the number of doses of vaccine (see the Methods section in the Supplementary Appendix). To assess the validity of reporting a constant efficacy per dose of vaccine, the number of doses was entered as a continuous variable and as a categorical variable in separate models, and these models were compared by means of a likelihood-ratio test. Variations in vaccine efficacy by zone and potential interactions between the vaccines were investigated. The sensitivity of the efficacy estimates to the matching criteria was also examined.

Vaccine coverage was estimated on the basis of the reported number of doses in cases of acute flaccid paralysis not caused by poliovirus, with cases weighted to match the underlying distribution of the population by state and single years of age.^{14,15} Differences in vaccine coverage between 2005 and 2007 were tested with the use of a multinomial chi-square test (see the Methods section in the Supplementary Appendix). The fraction of children younger than 5 years of age who were protected by direct vaccination against type 1 paralytic poliomyelitis (vaccine-induced population immunity) was calculated from the estimated coverage with monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine and their estimated efficacies (see the Methods section in the Supplementary Appendix). Confidence intervals were estimated with the use of the delta method (see the Methods section in the Supplementary Appendix). The contribution of changes in coverage only to changes in population immunity was estimated by recalculating these levels with the assumption that trivalent oral poliovirus vaccine was used in all rounds. The correlation between the proportion of children younger than 5 years of age who were protected by vaccination in each state and the reported numbers of cases of type 1 poliomyelitis was estimated with the use of Spearman's rank-correlation coefficient.

RESULTS

VACCINE EFFICACY

A total of 2532 children were reported to have type 1 poliomyelitis and 1092 were reported to

have type 3 poliomyelitis during the study period. Of these children, 1174 with type 1 poliomyelitis (46%) and 502 with type 3 poliomyelitis (46%) were matched with a suitable control (Table 1). The estimated efficacy of trivalent oral poliovirus vaccine against paralysis from type 1 poliomyelitis across Nigeria was 16% (95% confidence interval [CI], 10 to 21) per dose, and the efficacy of monovalent type 1 oral poliovirus vaccine was 67% (95% CI, 39 to 82) per dose. The estimated efficacy of trivalent oral poliovirus vaccine against paralysis from type 3 poliomyelitis was 18% (95% CI, 9 to 26) per dose. There was insufficient use of monovalent type 3 oral poliovirus vaccine to allow the efficacy of this vaccine to be estimated reliably.

Vaccine efficacies did not differ significantly according to the zone of the country (Table 1), nor were there significant differences when efficacies were estimated separately according to year or age (see Tables 3A and 3B in the Supplementary Appendix). There was no evidence of an interaction between monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine in the type 1 model ($P=0.82$). Comparisons of the fit of the model with a constant efficacy per dose of vaccine with a model that allowed the protective efficacy to vary according to the dose number showed that a constant efficacy per dose was a reasonable assumption for both monovalent type 1 oral poliovirus vaccine and trivalent oral poliovirus vaccine ($P=0.93$ and $P=0.085$, respectively, for monovalent and trivalent oral poliovirus vaccine against type 1 paralytic poliomyelitis; $P=0.36$ for trivalent oral poliovirus vaccine against type 3) (see Fig. 2 in the Supplementary Appendix). These findings are consistent with the all-or-nothing mode of action of these live-virus vaccines. Tighter matching of cases and controls according to age and date at the onset of paralysis did not substantially alter the estimates of vaccine efficacy (see Table 4 in the Supplementary Appendix). Sensitivity analyses showed that our results were robust with respect to various assumptions regarding the number of doses received through routine immunization (see Table 5 in the Supplementary Appendix).

The estimated sensitivity of laboratory testing for wild poliovirus was 99% and 98% for types 1 and 3, respectively. The prevalence of type 1 and type 3 poliovirus among cases of acute flaccid paralysis was estimated at 9.3% and 3.9%, respec-

Table 1. Estimated Protective Efficacy of Oral Poliovirus Vaccines against Type 1 and Type 3 Paralytic Poliomyelitis in Nigeria According to Zone, 2001–2007.

Zone	Protection against Type 1 Poliovirus				Protection against Type 3 Poliovirus		
	Total No. of Cases	Case–Control Matches	Efficacy of Monovalent Vaccine	Efficacy of Trivalent Vaccine	Total No. of Cases	Case–Control Matches	Efficacy of Trivalent Vaccine
		no. (%)	% (95% CI)			no. (%)	% (95% CI)
Northwest	1899	925 (49)	63 (27–82)	13 (6–19)	851	402 (47)	17 (7–26)
Northeast	437	158 (36)	58 (0–88)	23 (7–37)	173	67 (39)	18 (0–40)
North Central	154	68 (44)	100 (0–100)	23 (1–40)	52	16 (31)	24 (0–56)
South*	42	23 (55)	—†	54 (4–78)	16	8 (50)	12 (0–54)
All zones	2532	1174 (46)	67 (39–82)	16 (10–21)	1092	502 (46)	18 (9–26)

* Results for the South include all three southern zones (Southwest, South-South, and Southeast).

† During the study period, there were no reported cases of type 1 paralytic poliomyelitis in the South after the introduction of monovalent type 1 oral poliovirus vaccine.

tively. Therefore, the probability of misclassifying a case of acute flaccid paralysis not caused by poliovirus as a case of type 1 or type 3 poliomyelitis was 0.0011 and 0.0006, respectively.

IMMUNIZATION COVERAGE AND PROGRAM EFFECTIVENESS

Significant improvements in immunization coverage were registered between 2005 (before the administration of monovalent type 1 oral poliovirus vaccine and immunization-plus days) and 2007 (after the administration of monovalent type 1 oral poliovirus vaccine and immunization-plus days) in the northwestern and northeastern zones, which reported the majority of poliomyelitis cases ($P<0.001$) (Table 2). Coverage in the southeastern zone declined significantly between 2005 and 2007 ($P<0.001$) (Table 2). Coverage in the three remaining zones was high, and there were no significant changes between 2005 and 2007.

The combination of improved coverage and use of the more efficacious monovalent type 1 oral poliovirus vaccine has resulted in substantial increases in the proportion of children protected through direct immunization against type 1 paralytic poliomyelitis (Fig. 1 and Table 2). In the northwestern zone, 33% of the improvement in vaccine-induced immunity between 2005 and 2007 was due to improved coverage, whereas in the southern zones of the country, the improvement in vaccine-induced immunity was solely due to the introduction of the monovalent vaccine (see Tables 6 and 7 in the Supplementary Appendix).

The estimated fraction of children who were protected against type 1 paralytic poliomyelitis

strongly correlates with the reported numbers of cases of type 1 poliomyelitis (Fig. 1). The correlation was significant in all years, rising significantly in more recent years ($P=0.02$) (see Table 8 in the Supplementary Appendix). Plotting the fraction of children younger than 5 years of age who were protected from type 1 paralytic poliomyelitis against the incidence of poliomyelitis for each year and state suggests that in some areas a level of 80% protection has been sufficient to achieve local elimination (Fig. 2).

DISCUSSION

This study provides estimates of the efficacy of oral poliovirus vaccines in Nigeria and the effect of these vaccines on population immunity. Although trivalent oral poliovirus vaccine was successfully used to interrupt transmission of indigenous type 1 wild polioviruses across southern Nigeria, the recently licensed monovalent type 1 oral poliovirus vaccine, with an estimated efficacy per dose that is four times that of trivalent oral poliovirus vaccine, substantially improves the prospects for accelerating elimination in northern Nigeria. Because of improvements in immunization coverage and the introduction of monovalent type 1 oral poliovirus vaccine, vaccine-induced immunity in the northwestern and northeastern zones (which reported 96% of all type 1 cases in Nigeria in 2006) more than doubled between 2005 and 2007. However, further gains in immunization coverage are required to achieve elimination, since 21% of children in the northwestern zone (where the majority of cases have occurred)

Table 2. Vaccine Coverage among Children with Acute Flaccid Paralysis Not Caused by Poliovirus, According to Zone and Estimated Percentage of Children Less Than 5 Years of Age Who Were Protected against Type 1 Paralytic Poliomyelitis in 2005 and 2007.*

Year	Northwest	Northeast	North Central	Southwest	Southeast	South-South	Whole Country
2005							
No. of children	909	358	529	480	219	229	2724
<i>percentage of children</i>							
No. of reported doses							
0	41	28	4	2	2	1	15
1–3	44	42	30	28	39	31	37
>3	15	30	66	70	59	69	47
Children protected (95% CI)	22 (15–28)	31 (28–35)	49 (46–52)	56 (51–62)	50 (45–54)	53 (49–57)	42 (40–44)
2007							
No. of children	740	318	522	450	273	424	2727
<i>percentage of children</i>							
No. of reported doses							
0	21	9	3	1	7	3	9
1–3	55	59	38	27	46	31	43
>3	24	32	59	72	46	66	48
Children protected (95% CI)	56 (41–71)	61 (48–73)	65 (53–77)	83 (72–94)	71 (57–84)	81 (72–91)	69 (62–76)

* Distribution of the number of doses among case subjects differed significantly between 2005 and 2007 in the northwestern, northeastern, and southeastern zones and across the whole country ($P < 0.001$ for all four comparisons between the 2 years).

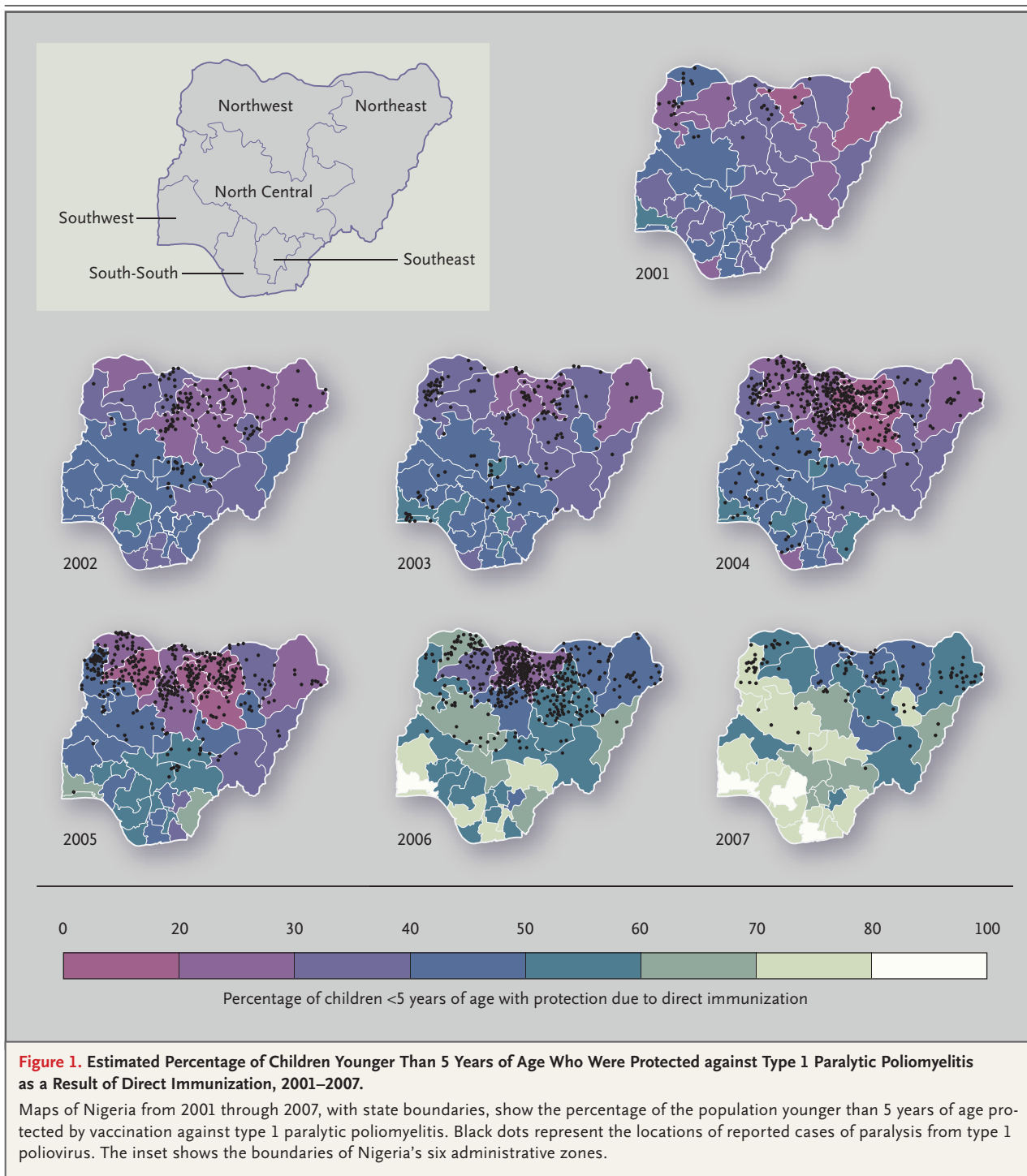
still have never received a single dose of vaccine, with a further 55% having received fewer than the recommended four doses.

The estimated efficacy of trivalent oral poliovirus vaccine in Nigeria is similar to its efficacy in India, with the exception of the northern Indian state of Uttar Pradesh, where the substantially lower efficacy of oral poliovirus vaccines appears to be the result of a high prevalence of diarrheal and other infections that interfere with seroconversion.¹⁶ The efficacy of trivalent oral poliovirus vaccine did not show significant variation across Nigeria, although case numbers in the South were limited (Table 1). The efficacy of monovalent type 1 oral poliovirus vaccine is higher in northern Nigeria than in Uttar Pradesh¹¹ and is similar to rates of seroconversion after administration of monovalent type 1 oral poliovirus vaccine in other developing countries.¹⁷ This finding suggests that the persistence of poliovirus in northern Nigeria is not a result of environmental conditions that can compromise vaccine efficacy, as it is in northern India.

The precision of our efficacy estimates could be affected by the accuracy of the reporting of oral poliovirus vaccine doses and potential differ-

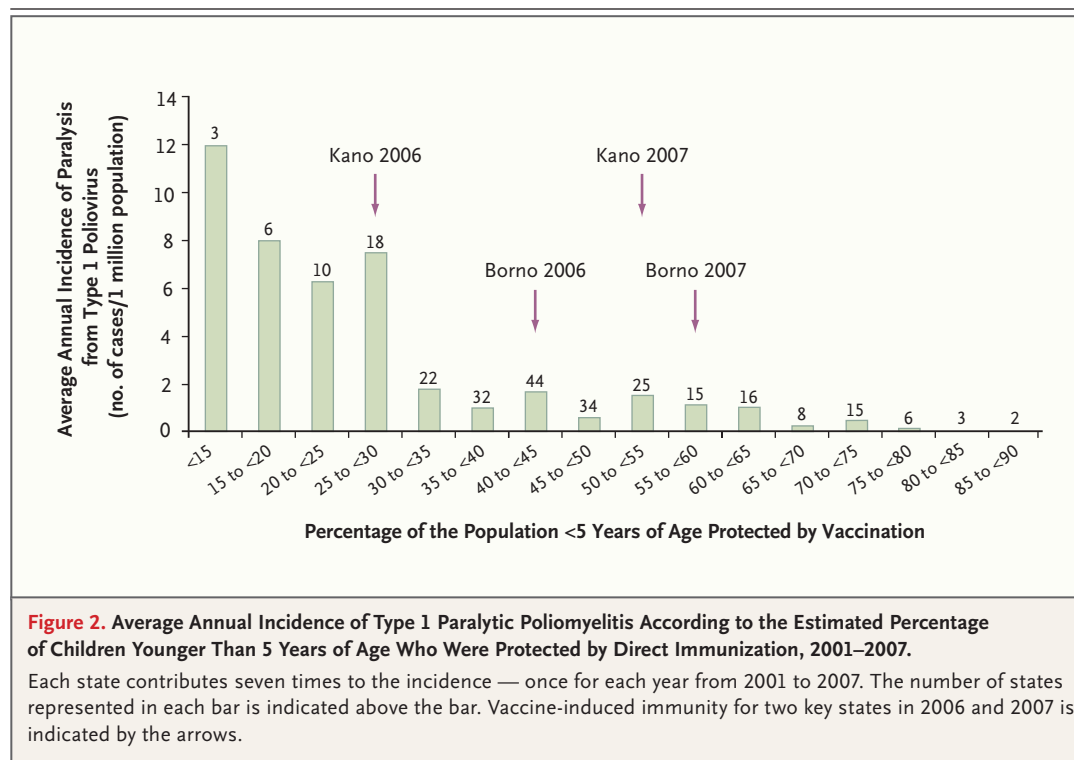
ences in exposure to poliovirus between cases and controls. However, the fact that the parent (or guardian) is unaware of the poliovirus status of a child when being interviewed minimizes the risk of systematic bias in dose reporting. In addition, since controls came from the same area as case subjects, the two groups are likely to have had similar exposure to virus; indeed, efficacy estimates were robust to closer matching of case subjects with controls. Nearly 50% of case subjects were matched to a control, and the resulting exclusion of some case subjects does not appear to have introduced any bias (see Tables 9A and 9B in the Supplementary Appendix). Perhaps more important, irrespective of the precision of the point estimate of vaccine efficacy, the calculated relative gain in efficacy for monovalent type 1 oral poliovirus vaccine as compared with trivalent oral poliovirus vaccine against type 1 paralytic poliomyelitis was robust with respect to these potential biases. Vaccine quality is unlikely to have been an issue, since temperature monitors on vaccine vials have been used nationwide since 2000 to prevent the administration of vaccine with low potency.

Our estimates of population immunity do not



account for secondary transmission of vaccine virus, maternal antibodies in the first few months of life, or natural immunity from exposure to circulating wild poliovirus. However, they do allow a direct comparison of vaccine-induced immunity

between states that have had supplementary immunization activities with different types of vaccine and variable frequency and coverage, thereby providing a good relative indication of program effectiveness. The improvements observed in vac-



cine coverage indicate that increases in immunity levels in northwestern Nigeria are not purely a result of the higher efficacy of monovalent type 1 oral poliovirus vaccine. Many factors probably contributed, including the introduction of immunization-plus days and other such efforts by field staff and local and national leaders to address the concerns of community leaders and parents that had contributed to the suspension of the use of oral poliovirus vaccine in 2003.

The encouraging findings of this study must be interpreted with caution in assessing the overall outlook for the elimination of poliovirus in Nigeria. With substantial population movements and an annual birth rate of approximately 40 births per 1000 population,¹⁴ the growth of the susceptible population is considerable. Although a level of direct immunity from vaccination of approximately 30% can reduce the transmission of type 1 poliovirus in Nigeria (Fig. 2), a level of at least 80% is required for sustained local elimination of transmission. As of 2007, some degree of control appears to have been achieved in many northern states, but considerable further improvements are still required to reach a level at which poliovirus can be eradicated (Fig. 1). The consequences of such ongoing immunity gaps were evi-

dent in the recent emergence of a circulating vaccine-derived poliovirus in the North¹⁸ and a resurgence of type 1 cases in early 2008⁸ that have spread to the South. Nonetheless, giving each child in the northwestern zone just one additional dose of monovalent type 1 oral poliovirus vaccine could increase direct population immunity to more than 80% in much of this area — a substantial increase from the 56% immunity recorded in 2007.

India and Nigeria reported 88% of poliomyelitis cases worldwide in 2007 and are central to global eradication. In contrast with India, in northern Nigeria vaccine coverage remains a significant barrier to completing the elimination of poliovirus. The relatively high efficacy of monovalent type 1 oral poliovirus vaccine in Nigeria offers the potential for rapid eradication of type 1 poliovirus, provided that vaccination coverage continues to improve in the northern part of the country.

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We thank all those involved in acute-flaccid-paralysis surveillance and laboratory testing in Nigeria.

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ORIGINAL ARTICLE

Implications of a Circulating Vaccine-Derived Poliovirus in Nigeria

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ABSTRACT

BACKGROUND

The largest recorded outbreak of a circulating vaccine-derived poliovirus (cVDPV), detected in Nigeria, provides a unique opportunity to analyze the pathogenicity of the virus, the clinical severity of the disease, and the effectiveness of control measures for cVDPVs as compared with wild-type poliovirus (WPV).

METHODS

We identified cases of acute flaccid paralysis associated with fecal excretion of type 2 cVDPV, type 1 WPV, or type 3 WPV reported in Nigeria through routine surveillance from January 1, 2005, through June 30, 2009. The clinical characteristics of these cases, the clinical attack rates for each virus, and the effectiveness of oral polio vaccines in preventing paralysis from each virus were compared.

RESULTS

No significant differences were found in the clinical severity of paralysis among the 278 cases of type 2 cVDPV, the 2323 cases of type 1 WPV, and the 1059 cases of type 3 WPV. The estimated average annual clinical attack rates of type 1 WPV, type 2 cVDPV, and type 3 WPV per 100,000 susceptible children under 5 years of age were 6.8 (95% confidence interval [CI], 5.9 to 7.7), 2.7 (95% CI, 1.9 to 3.6), and 4.0 (95% CI, 3.4 to 4.7), respectively. The estimated effectiveness of trivalent oral polio vaccine against paralysis from type 2 cVDPV was 38% (95% CI, 15 to 54%) per dose, which was substantially higher than that against paralysis from type 1 WPV (13%; 95% CI, 8 to 18%), or type 3 WPV (20%; 95% CI, 12 to 26%). The more frequent use of serotype 1 and serotype 3 monovalent oral polio vaccines has resulted in improvements in vaccine-induced population immunity against these serotypes and in declines in immunity to type 2 cVDPV.

CONCLUSIONS

The attack rate and severity of disease associated with the recent cVDPV identified in Nigeria are similar to those associated with WPV. International planning for the management of the risk of WPV, both before and after eradication, must include scenarios in which equally virulent and pathogenic cVDPVs could emerge.

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SINCE 1988, WHEN THE WORLD HEALTH Assembly resolved to eradicate poliovirus, the annual incidence of paralytic poliomyelitis has fallen by more than 99%. However, the annual number of cases reported for the years 2003 through mid-2009 has remained relatively constant, and continued transmission in endemic countries, especially Nigeria, led to a resurgence of re-infected countries across Africa from 2008 through 2009.^{1,2} The emergence of a serotype 2 circulating vaccine-derived poliovirus (cVDPV) in Nigeria has complicated the epidemiology of polio as well as vaccine selection and scheduling for supplementary immunization activities.³

A cVDPV is defined by the appearance of two or more cases of acute flaccid paralysis with closely related vaccine-derived poliovirus; it occurs when a vaccine poliovirus increases in neurovirulence and transmissibility after mutation.¹ cVDPVs have usually recombined with other enteroviruses, and their spread is associated with inadequate vaccine coverage.⁴ Globally, 12 independent outbreaks of cVDPV have been identified to date.^{1,4,5} However, little is known about their clinical features or the challenges they pose for eradication. Consequently, their significance has been much debated, with some scientists arguing that cVDPVs are the death knell for eradication^{6,7} and others suggesting that these viruses pose a minor risk.⁸

Serotype 2 cVDPVs are of particular concern because serotype 2 wild-type poliovirus (WPV) is considered to have been eradicated.⁹ The ongoing outbreak of paralytic disease associated with this type 2 cVDPV in Nigeria is unique in being the largest recorded to date and in occurring in the presence of extensive transmission of indigenous type 1 and type 3 WPV. Therefore, steps have been taken to intersperse the administration of trivalent oral polio vaccine — the only vaccine currently licensed in Nigeria that provides protection against type 2 WPV — among more aggressive efforts to administer the more effective serotype 1 or 3 monovalent oral polio vaccines in order to limit the national and international spread of WPVs.¹⁰⁻¹³

Despite the fact that both large- and small-scale campaigns using trivalent oral polio vaccine were mounted from 2006 through 2008, the number of cases of acute flaccid paralysis associated with type 2 cVDPV increased in early 2009. To optimize the balance of trivalent, monovalent, and the recently developed bivalent (serotypes

1 and 3) oral polio vaccines¹⁴ in upcoming supplementary immunization activities in Nigeria, and to appropriately plan for risk management after eradication, we need a deeper understanding of the relative pathogenicity of the cVDPV and WPVs, the relative clinical severity of the paralytic polio caused by the viruses, and the relative effectiveness of the control strategies currently being deployed against them.

METHODS

DATA COLLECTION

Intensified surveillance for poliomyelitis involving the detection and reporting of cases of acute flaccid paralysis¹⁵ among all children in Nigeria younger than 15 years of age began in December 1996.¹⁶ Case investigation involves conducting detailed clinical and epidemiologic investigations, including the collection of two stool samples for laboratory testing for poliovirus. Samples with positive results are investigated with the use of intratypic differentiation tests and genetic sequencing (carried out by the U.S. Centers for Disease Control and Prevention) to determine whether the virus is vaccine-related or wild-type.¹⁷ The number of doses of oral polio vaccine received before the development of acute flaccid paralysis is recorded during initial interviews with a senior medical officer. A follow-up visit at 60 days includes testing for residual paralysis. Clinical data collected during the initial interview include age, sex, presence or absence of asymmetrical paralysis, progression to paralysis within 3 days or over a longer period, presence of fever at onset or absence of paralysis, and number of limbs paralyzed.

We defined a case of cVDPV paralysis as any case of acute flaccid paralysis in which vaccine-related poliovirus isolated from at least one stool sample differed from the original Sabin vaccine strain by 1 to 15% of nucleotides in the region encoding the viral capsid protein VP1.¹⁸ Cases of wild-type poliomyelitis virus were defined as cases of acute flaccid paralysis in which the wild-type virus was detected in at least one stool sample. Cases of nonpolio acute flaccid paralysis were defined as those in which neither wild-type nor vaccine-related poliovirus was isolated in two adequate stool samples collected within 2 weeks after the onset of paralysis, at least 24 hours apart. All cases of acute flaccid paralysis with a reported date of onset between January 1, 2005, and June 30, 2009, were included in this analy-

sis. Cases without two adequate stool samples and with residual paralysis (assessed at least 60 days after the onset of paralysis) that were compatible with poliomyelitis were excluded, as were cases in which more than one serotype of WPV was isolated. Estimates of vaccine effectiveness and population immunity also excluded cases of acute flaccid paralysis in which the number of oral polio vaccine doses received or the age at onset of paralysis were not reported.

Most doses of oral polio vaccine received by children in Nigeria are delivered through supplementary immunization activities rather than routine services. The number of doses of each type of oral polio vaccine received by children with acute flaccid paralysis was therefore estimated on the basis of the reported total number of oral polio vaccine doses they received and the type of vaccine they were exposed to through supplementary immunization activities, calculated on the basis of their age and district of residence (for details, see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org).

STATISTICAL ANALYSIS

The clinical characteristics of acute flaccid paralysis associated with isolation of type 1 WPV, type 3 WPV, and type 2 cVDPV were compared with the use of Fisher's exact test (for categorical variables) or Student's *t*-test (for age, allowing for unequal variances).

Clinical attack rates among districts reporting all three types of poliovirus during the study period were estimated on the basis of the annual incidence of acute flaccid paralysis associated with each poliovirus per 100,000 children younger than 5 years of age and per 100,000 children younger than 5 years of age who were estimated to be unprotected by vaccination against the relevant serotype (for details, see the Methods section in the Supplementary Appendix). For example, 10 cases of type 1 WPV poliomyelitis isolated from a population of 100,000 children with 80% vaccine-induced immunity against serotype 1 would represent a clinical attack rate of 10 cases per 100,000 children and 50 per 100,000 susceptible children. Bayesian confidence intervals for the clinical attack rate per 100,000 susceptible children were calculated with the use of a hierarchical model (for details, see the Methods section in the Supplementary Appendix).

The effectiveness per dose of the different oral

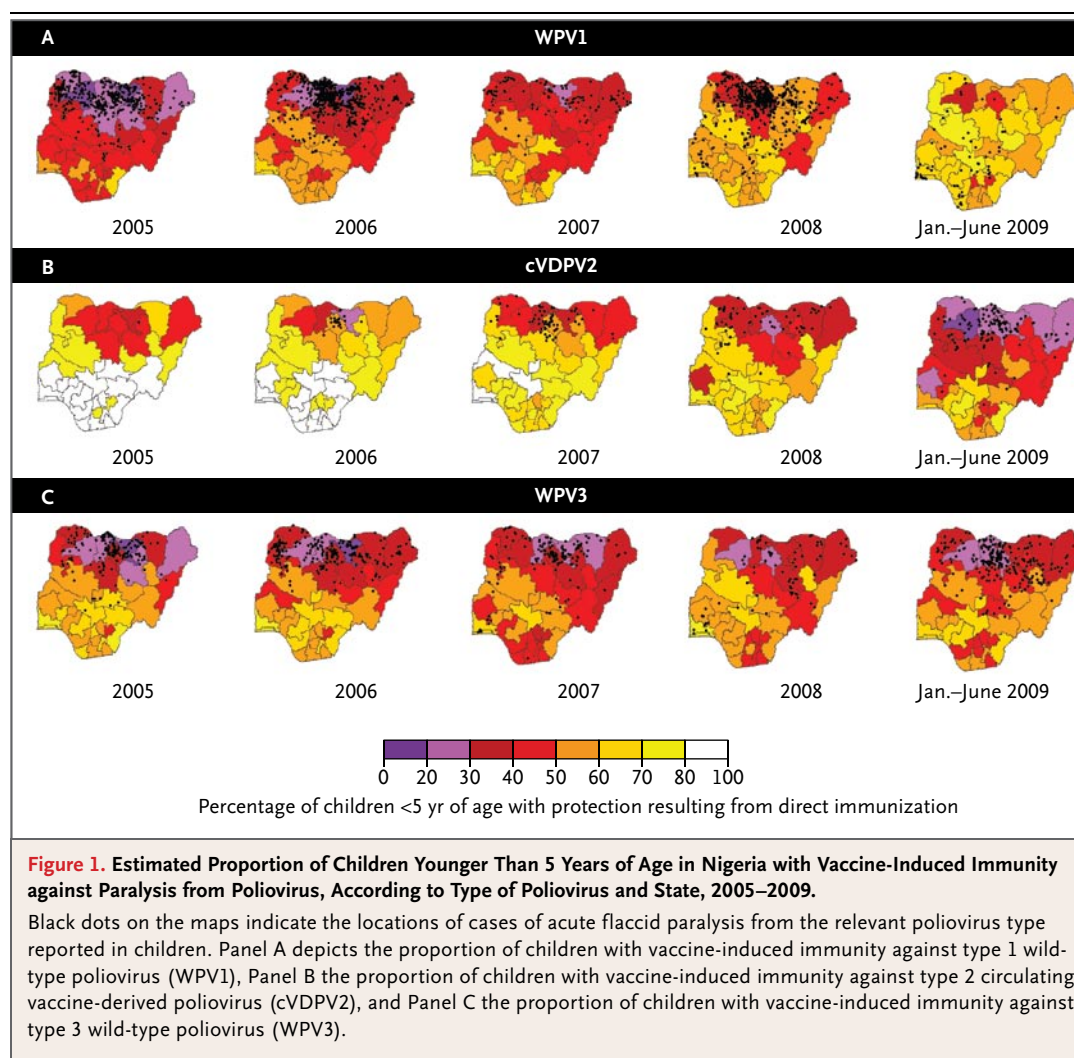
polio vaccines in providing protection against paralysis from each poliovirus was estimated with the use of a matched (1:1) case-control approach as described previously^{11,12,19} (for details, see the Methods section in the Supplementary Appendix). Population immunity from paralysis induced by means of direct immunization with oral polio vaccine among children under 5 years of age was estimated according to state and year on the basis of reports of the number of oral polio vaccine doses received by children with nonpolio acute flaccid paralysis and the estimated effectiveness of the vaccines.^{11,12} Estimates were weighted by single years of age to reflect the underlying age distribution of the population,²⁰ and estimates by zone were weighted to reflect the underlying population distribution by state.²¹ The effect of routine immunization coverage with trivalent oral polio vaccine on population immunity was assessed on the basis of coverage reported by the National Immunization Coverage Survey, with a hierarchical model used to assign trivalent oral polio vaccine doses to cases of nonpolio acute flaccid paralysis (see the Methods section in the Supplementary Appendix). Changes in population immunity were assessed by fitting a linear trend to the yearly estimates, with each year weighted by the sample size.

Monthly estimates of the hazard of a district reporting its first case of acute flaccid paralysis caused by type 2 cVDPV were examined as a function of variables representing the force of infection from cases reported in the previous 6 months, vaccine-induced population immunity, population size, and population density. These values were entered as time-varying covariates with the use of proportional-hazards regression, and the final model was chosen on the basis of maximum likelihood (see the Methods section in the Supplementary Appendix). The proportional-hazards assumption was assessed with the use of Schoenfeld's global test.

RESULTS

SEVERITY OF DISEASE AND CLINICAL ATTACK RATES

A total of 23,004 children were reported to have acute flaccid paralysis over the course of the study period. Among these children, 278 had type 2 cVDPV in their stool, 2323 had type 1 WPV, 1059 had type 3 WPV, and 2 had both type 1 and type 3 WPV (Fig. 1, and Fig. 1 and 2 and Table 1, Sections A through C, in the Supplementary Appen-



dix). The cases of acute flaccid paralysis with type 2 cVDPV were broadly similar to those with type 1 WPV or type 3 WPV in terms of the distribution of age and sex. There were no significant differences in the severity of clinical disease (Table 1).

In districts reporting all three types of poliovirus, the estimated clinical attack rates (measured according to annual incidence) of paralytic polio per 100,000 susceptible children under 5 years of age were 6.8 for type 1 WPV, 2.7 for type 2 cVDPV, and 4.0 for type 3 WPV (Table 2).

VACCINE EFFECTIVENESS

The estimated effectiveness of a dose of trivalent oral polio vaccine was greater against paralysis resulting from infection with type 2 cVDPV than against paralysis from type 1 WPV ($P=0.04$) and type 3 WPV ($P=0.12$) (Table 2 and Fig. 2). Sero-

type 1 monovalent oral polio vaccine was significantly more protective than trivalent oral polio vaccine against paralysis from type 1 WPV ($P<0.001$), and serotype 3 monovalent oral polio vaccine was nonsignificantly more protective than trivalent oral polio vaccine against paralysis from type 3 WPV ($P=0.08$). A regression model with a variable effectiveness per dose of oral polio vaccine did not provide a significantly better fit than a model with constant effectiveness per dose (P values for all vaccine and poliovirus types are available in Fig. 3 in the Supplementary Appendix).

The majority of cases of poliomyelitis were matched to a suitable control. There were no substantial differences in vaccine effectiveness across the country or when effectiveness was estimated separately according to year or age. For instances in which some significant interactions were noted

Table 1. Characteristics of Children with Acute Flaccid Paralysis and Type 1 Wild-Type Poliovirus, Type 2 Circulating Vaccine-Derived Poliovirus, or Type 3 Wild-Type Poliovirus, Isolated from Stool.*

Variable	Type 1 WPV (N=2323)	Type 2 cVDPV (N=278)	Type 3 WPV (N=1059)	P Value†	
				Type 2 cVDPV vs. Type 1 WPV	Type 2 cVDPV vs. Type 3 WPV
Sex — no. (%)					
Male	1312 (56)	172 (62)	609 (58)	0.04	0.11
Female	1010 (43)	101 (36)	449 (42)		
Missing data	1 (<1)	5 (2)	1 (<1)		
Mean age — yr	2.36±0.03	2.57±0.10	2.44±0.05	0.04	0.74
Missing data — no. (%)	255 (11)	35 (13)	87 (8)		
Asymmetric paralysis — no. (%)					
Yes	1719 (74)	209 (75)	779 (74)	0.37	0.34
No	570 (25)	60 (22)	265 (25)		
Missing data	34 (1)	9 (3)	15 (1)		
Progression to paralysis within 3 days — no. (%)					
Yes	2278 (98)	269 (97)	1038 (98)	>0.99	>0.99
No	25 (1)	3 (1)	13 (1)		
Missing data	20 (1)	6 (2)	8 (1)		
Fever at onset — no. (%)					
Yes	2286 (98)	269 (97)	1039 (98)	>0.99	0.75
No	18 (1)	2 (1)	13 (1)		
Missing data	19 (1)	7 (3)	7 (1)		
No. of limbs affected — no. (%)					
0	11 (<1)	0 (0)	9 (1)	0.06	0.17
1	1197 (52)	142 (51)	567 (54)		
2	996 (43)	104 (37)	423 (40)		
3	68 (3)	10 (4)	35 (3)		
4	44 (2)	12 (4)	23 (2)		
Missing data	7 (<1)	10 (4)	2 (<1)		
Residual paralysis at 60-day follow-up — no. (%)					
Yes	541 (23)	129 (46)	370 (35)	0.12	0.06
No	98 (4)	33 (12)	60 (6)		
Lost to follow-up	2 (<1)	1 (<1)	3 (<1)		
Dead at follow-up	4 (<1)	1 (<1)	6 (1)		
Missing data	1678 (72)	114 (41)	620 (59)		

* Plus-minus values are means ±SE. Percentages may not total 100 because of rounding. cVDPV denotes circulating vaccine-derived poliovirus, and WPV wild-type poliovirus.

† Analyses excluded children with missing data and those lost to follow-up or dead at follow-up.

between vaccine effectiveness and location or year (for serotype 1 only), matching on the basis of district rather than state resolved this bias (which was the result of supplementary immunization activities at the district level rather than the state level), and overall estimates of vaccine effectiveness were unchanged (see Table 3, Sections A and B, in the Supplementary Appendix). Tighter matching of cases and controls (on the basis of age, date, and residence) did not substantially alter the estimates of vaccine effectiveness. Although matching on the basis of district rather than state

Table 2. Estimated Effectiveness against Paralysis per Dose of Oral Polio Vaccine, According to the Types of Polioviruses Circulating in Nigeria and the Estimated Annual Rates of Clinical Attack.*

Variable	WPV1 (N=2323)	cVDPV2 (N=278)	WPV3 (N=1059)
Per-dose vaccine effectiveness			
Cases matched to controls — no. (%)	1739 (75)	184 (66)	812 (77)
Monovalent oral polio vaccine 1 — % (95% CI)	43 (33–51)	NA†	NA†
Trivalent oral polio vaccine — % (95% CI)	13 (8–18)	38 (15–54)	20 (12–26)
Monovalent oral polio vaccine 3 — % (95% CI)	NA†	NA†	53 (16–74)
Annual clinical attack rate			
No. of cases among children <5 yr old‡	1075	211	508
No./100,000 susceptible children <5 yr old — (95% CI)	6.8 (5.9–7.7)	2.7 (1.9–3.6)	4.0 (3.4–4.7)
No./100,000 children <5 yr old, susceptible and vaccinated combined — (95% CI)	5.3 (4.9–5.6)	1.5 (1.3–1.6)	3.2 (2.8–3.5)

* CI denotes confidence interval, cVDPV circulating vaccine-derived poliovirus, NA not applicable, and WPV wild-type poliovirus.

† The vaccine does not provide protection against this type of polio.

‡ This category represents districts that reported cases of acute flaccid paralysis resulting from each of three poliovirus types during the study period.

increased the estimate of vaccine effectiveness against type 2 cVDPV to 54% per dose, only 21% of cases were matched, which led to wide confidence intervals. Consequently, matching by state was preferable, although it may have slightly underestimated overall vaccine effectiveness.

Sensitivity analyses showed that our results were reasonably robust to varying assumptions regarding the number of doses received through routine immunization and that trivalent oral polio vaccine was consistently more effective against paralysis from type 2 cVDPV than against paralysis from type 1 or type 3 WPV (see Table 4, Sections A through C, in the Supplementary Appendix).

VACCINE-INDUCED POPULATION IMMUNITY

Estimated vaccine-induced population immunity to type 1 WPV poliomyelitis increased significantly in all zones from 2005 to 2009, particularly in the North West and North East zones (by 7.4% and 8.1%, respectively, annually) (Fig. 1). However, estimated vaccine-induced population immunity to type 2 cVDPV poliomyelitis declined significantly during the study period in all zones (range, –4.7% to –8.8% annually). Estimated vaccine-induced population immunity to type 3 WPV poliomyelitis increased significantly (by approximately 1% annually) in the North West and North East zones and declined significantly in two of the other four zones. Annual estimates for each serotype

and results of the tests for trends over time in each zone are given in Table 5 in the Supplementary Appendix.

INCIDENCE OF cVDPV

The final proportional-hazards model for a district reporting its first case of paralysis associated with type 2 cVDPV included a function accounting for the distance from other reported cases of type 2 cVDPV paralysis, vaccine-induced population immunity (according to state) in the previous 6 months, and an interaction term between these two variables (Fig. 3, and Fig. 4 and Table 6 in the Supplementary Appendix). This model suggests a strongly localized risk associated with the well-defined geographic spread of this virus. The interaction term indicates that increases in immunity levels were associated with a greater proportional reduction in the hazard when fewer cases had been reported nearby. Variables based on population numbers or density were excluded from the final model, since they were not significant and did not confound any other variables in the model substantially. The final model satisfied the assumption of proportional hazards ($P=0.25$).

DISCUSSION

This study shows that in Nigeria the pathogenicity of type 2 cVDPV and the severity of paralytic disease resulting from infection are similar to that

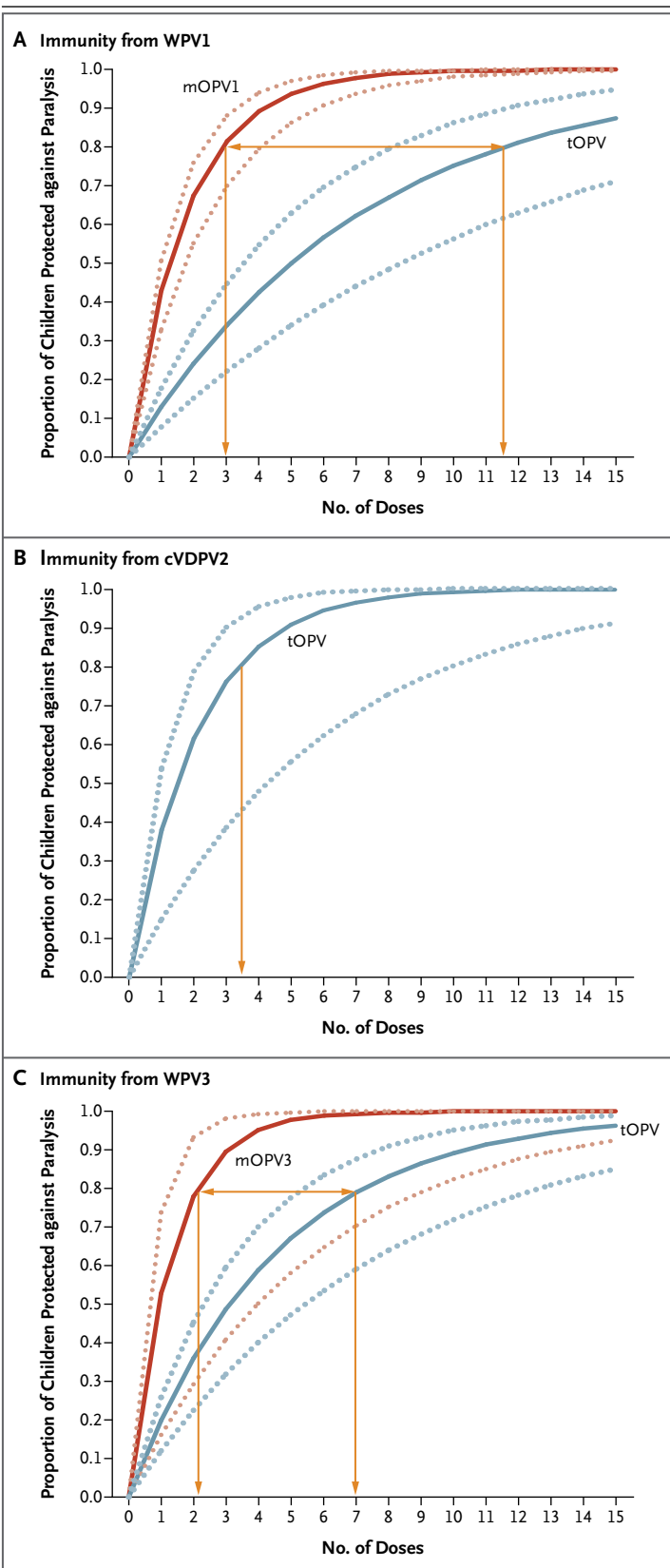


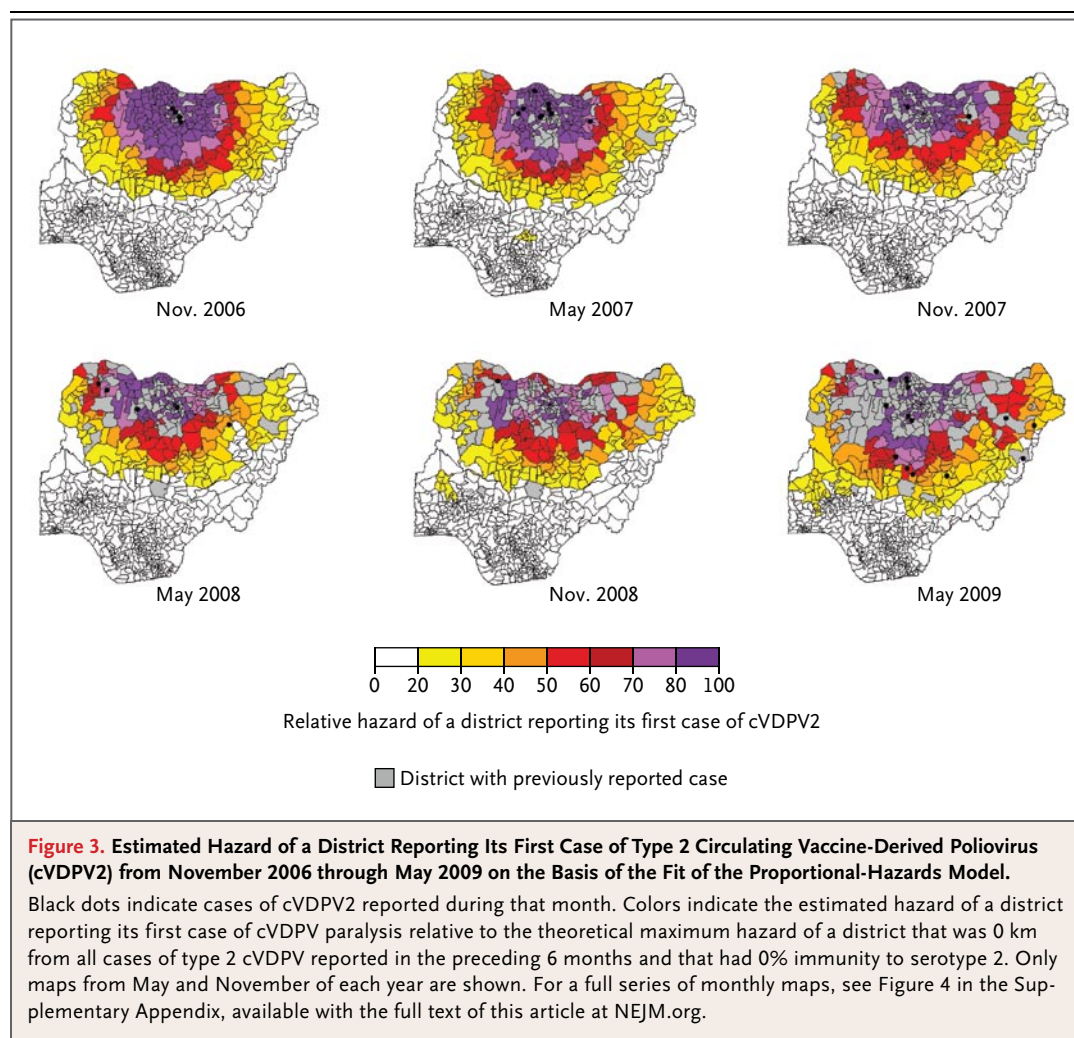
Figure 2. Predicted Proportion of Children Protected against Paralysis, According to Oral Poliovirus Vaccine Type and Number of Doses.

Data are based on the estimated effectiveness per dose. Solid red lines indicate monovalent oral vaccines against type 1 and type 3 polioviruses (mOPV1 and mOPV3, respectively) and solid blue lines the trivalent vaccine (tOPV). Dotted lines indicate 95% confidence intervals. The number of doses required for 80% protection are shown with arrows for purposes of illustration and of comparing the effectiveness of the monovalent vaccine with that of the trivalent vaccine. Panel A depicts the proportion of children with protection against type 1 wild-type poliovirus (WPV1), Panel B the proportion with protection against type 2 circulating vaccine-derived poliovirus (cVDPV2), and Panel C the proportion of children with protection against type 3 wild-type poliovirus (WPV3).

of WPVs. This study also provides an estimate of the effectiveness of an oral polio vaccine against paralysis from infection with a cVDPV and indicates that trivalent oral polio vaccine provides greater protection against infection with type 2 cVDPV than against infection with type 1 WPV or type 3 WPV. This finding is consistent with the higher rates of seroconversion to this serotype observed after administration of trivalent oral polio vaccine in developing countries.²² Our results indicate that interrupting the transmission of a cVDPV can require control measures and levels of population immunity similar to those needed to interrupt an outbreak caused by WPV.

The equivalent severity of disease caused by cVDPVs and WPVs is consistent with the common mechanism of disease responsible for poliovirus-associated paralysis.²³ In districts where type 2 cVDPV and WPV cocirculated, the clinical attack rate for type 2 cVDPV was only slightly lower than that for type 1 and type 3 WPV, which is consistent with the lower rate of clinical attack for type 2 WPV than for type 1 WPV.²⁴ This observation was made despite the recent introduction and spread of type 2 cVDPV, suggesting that both the pathogenicity and transmissibility of type 2 cVDPV are similar to those of type 2 WPV. A study of serotype 1 cVDPV in Indonesia (45 cases), where there was limited cocirculation of type 1 WPV (8 cases), also suggested similar attack rates for these WPVs and cVDPVs.²⁵

Although the 11 previously recognized outbreaks of cVDPV were terminated after one to four immunization campaigns had been conducted with either trivalent oral polio vaccine or sero-



type-specific monovalent oral polio vaccine,⁴ the outbreak in Nigeria persisted in spite of six large-scale and several smaller-scale campaigns carried out with the use of trivalent oral polio vaccine. Given the relatively high estimated effectiveness of trivalent oral polio vaccine against type 2 cVDPV, its persistence can be attributed to inadequate coverage with this vaccine during supplementary immunization activities. Furthermore, the six large campaigns were conducted over the course of 4 years and were interspersed with several rounds of immunization with monovalent oral polio vaccine to control infection with WPVs. By 2009, persistent coverage gaps during supplementary immunization activities, combined with low levels of routine immunization, resulted in a further widening of the immunity gap and the upsurge in the cases of type 2 cVDPV observed

in the first half of 2009. The marked drop in cases of poliomyelitis associated with type 2 cVDPV in the second half of 2009 (10 cases from July through December vs. 138 cases from January through June) after the implementation of just two large-scale campaigns with trivalent oral polio vaccine of moderate quality in May and August is consistent with this vaccine's high level of effectiveness against paralysis from type 2 cVDPV. This finding bodes well for our ability to interrupt transmission of this virus.

We have previously discussed the limitations of estimating vaccine effectiveness and population immunity from reported doses of oral polio vaccine received by children with acute flaccid paralysis.^{11,12,19} Although these limitations may affect the accuracy of our estimates, the finding of a greater protective effect of trivalent oral polio

vaccine against type 2 cVDPV than against type 1 or type 3 WPV and the better performance of the monovalent oral polio vaccines are robust to these limitations. Furthermore, our estimates of population immunity are predictive of the epidemiology of type 2 cVDPV. Since children with polio are less likely than control children to have received the more effective vaccine against the polio serotype with which they were infected, a case-control approach based on the inferred type of vaccine received can underestimate the effectiveness of monovalent oral polio vaccines. Despite this potential bias, the estimated effectiveness of monovalent oral polio vaccines was high as compared with that of the trivalent oral polio vaccine and provides a conservative estimate of the effectiveness of these vaccines as compared with trivalent oral polio vaccine.

Although we did not account for the number of asymptomatic infections associated with polioviruses in our hazard analysis, we made the reasonable assumption that the number of children with acute flaccid paralysis who had poliovirus in their stool was proportional to the number of infections with this virus. In addition, although four lineages of circulating serotype 2 VDPV emerged during the study period, the current outbreak is largely the result of a single lineage (more than 90% of cases).³ Our estimates of vaccine-induced immunity do not account for exposure to oral polio vaccine excreted by vaccinees, the presence of maternal antibodies early in life, or asymptomatic infection with circulating polioviruses. These factors may affect estimates of the attack rates among susceptible children. However, estimates of the attack rates among all children are consistent with our finding of only slightly lower rates for type 2 cVDPV (Table 2).

These findings have important short-term and long-term implications for the Global Polio Eradication Initiative. First, this analysis supports the

strengthening of current recommendations that responses to outbreaks of cVDPVs should be aligned with responses to outbreaks of WPVs. Second, although state-level supplementary immunization activities have been sufficient to interrupt the spread of cVDPVs in most settings, the model shown in Figure 3 indicates that additional, large-scale rounds of immunization with trivalent oral polio vaccine, such as those conducted in May and August 2009, are required across northern Nigeria. Finally, the Global Polio Eradication Initiative's plans for managing risks after the eradication of WPV must include scenarios in which transmissible and pathogenic cVDPVs could emerge, particularly immediately after the worldwide cessation of the use of oral polio vaccine.²⁶

The eradication of type 2 WPV globally in 1999 was a remarkable achievement. However, this study points out the fragility of that achievement, given the pathogenicity and severity of disease that can be associated with type 2 cVDPV. Fortunately, trivalent oral polio vaccine is highly protective against this cVDPV, and modest gains in coverage now appear to be facilitating its rapid interruption. The steep decline in WPV cases in northern Nigeria during the second half of 2009 and the concomitant declines in cases of infection with type 2 cVDPV that followed improvements in coverage through supplementary immunization activities,²⁷ together with the potential role of recently licensed bivalent oral polio vaccine,²⁸ improve the prospects for the eradication of these polioviruses in Nigeria.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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The Duration of the Effects of Repeated Widespread Badger Culling on Cattle Tuberculosis Following the Cessation of Culling

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Abstract

Background: In the British Isles, control of cattle tuberculosis (TB) is hindered by persistent infection of wild badger (*Meles meles*) populations. A large-scale field trial—the Randomised Badger Culling Trial (RBCT)—previously showed that widespread badger culling produced modest reductions in cattle TB incidence during culling, which were offset by elevated TB risks for cattle on adjoining lands. Once culling was halted, beneficial effects inside culling areas increased, while detrimental effects on adjoining lands disappeared. However, a full assessment of the utility of badger culling requires information on the duration of culling effects.

Methodology/Principal Findings: We monitored cattle TB incidence in and around RBCT areas after culling ended. We found that benefits inside culled areas declined over time, and were no longer detectable by three years post-culling. On adjoining lands, a trend suggesting beneficial effects immediately after the end of culling was insignificant, and disappeared after 18 months post-culling. From completion of the first cull to the loss of detectable effects (an average five-year culling period plus 2.5 years post-culling), cattle TB incidence was 28.7% lower (95% confidence interval [CI] 20.7 to 35.8% lower) inside ten 100 km² culled areas than inside ten matched no-culling areas, and comparable (11.7% higher, 95% CI: 13.0% lower to 43.4% higher, $p = 0.39$) on lands ≤ 2 km outside culled and no-culling areas. The financial costs of culling an idealized 150 km² area would exceed the savings achieved through reduced cattle TB, by factors of 2 to 3.5.

Conclusions/Significance: Our findings show that the reductions in cattle TB incidence achieved by repeated badger culling were not sustained in the long term after culling ended and did not offset the financial costs of culling. These results, combined with evaluation of alternative culling methods, suggest that badger culling is unlikely to contribute effectively to the control of cattle TB in Britain.

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Introduction

Public controversy surrounds efforts to control bovine tuberculosis (TB) in the British Isles. Although bovine TB's causative agent (*Mycobacterium bovis*) primarily affects cattle, other mammalian hosts can be infected, including humans [1] and a number of wildlife species [2]. In the British Isles, control of cattle TB has been hampered by transmission of infection from wild badgers (*Meles meles*), and various forms of badger culling have been implemented to try to reduce such transmission [3]. Despite these efforts, the incidence of cattle TB remains high in both Britain and Ireland [4,5], with 2,738 confirmed herd breakdowns in Britain in 2008 [4] and national expenditure of over £100 million. This situation has provoked heated debate as cattle TB can profoundly affect farmers' livelihoods, yet culling of badgers – which are nationally protected in the UK by their own Act of Parliament (http://www.opsi.gov.uk/ACTS/acts1992/ukpga_19920051_en_1) – is unpopular with the general public [6].

In 1998, the UK government launched a large-scale field trial (the Randomised Badger Culling Trial, RBCT) to assess the potential contribution of badger culling to the control of cattle TB [7]. The incidence of cattle TB in and around 10 large (100 km²) areas subjected to annual badger culling was compared with that in and around 10 matched areas with no such culling. While culling was ongoing, it was associated with a modest reduction in the incidence of cattle TB inside culled areas; however this beneficial effect was almost cancelled out by an increase in cattle TB incidence on adjoining uncultured land [8,9]. These simultaneous beneficial and detrimental effects meant that, over the five-year culling period, the financial costs of conducting any form of culling far outweighed the savings achieved through reductions in the numbers of cattle herds experiencing TB breakdowns [8,10].

In the two years after culling ended, however, greater benefits became apparent: the positive effects inside culled areas became more pronounced, while the detrimental effects on adjoining land were no longer apparent [11]. Nevertheless, at that time the numbers of breakdowns prevented during and after culling were still not sufficient to offset the financial costs of conducting the culls [11]. Informed by these findings, and considering other factors such as practicality and public acceptability, the Secretary of State for Environment decided against badger culling to control cattle TB in England (<http://www.defra.gov.uk/news/2008/080707b.htm>). However, the Welsh Assembly Government proposes to implement a badger cull using methods to identify culling areas, and to cull badgers, very similar to those used in the RBCT (<http://www.wales.gov.uk/bovinetb>; though it faces a legal challenge to this proposal http://www.badger.org.uk/_Attachments/Resources/326_S4.pdf). Culling is also being considered in Northern Ireland (<http://www.dardni.gov.uk/tb-statement.pdf>).

The cost-effectiveness of badger culling as a cattle TB control measure depends in part on the duration of the benefits it imparts. If the effects are long-lasting, then the long-term benefits (in terms of breakdowns prevented) might offset the medium-term costs (in terms of the financial costs of culling, as well as the additional breakdowns on adjoining land prompted by culling). Here, we use updated cattle TB incidence data from RBCT areas to determine the duration of the effects of repeated widespread badger culling on cattle TB following the cessation of culling.

Methods

Data presented here come from RBCT areas subjected to proactive culling (widespread culling, repeated approximately annually) and from their matched no-culling controls. RBCT methods are described in detail in refs [9] and [10] but, in brief, thirty 100km² RBCT areas, arranged as 10 “triplets”, were selected in areas of England with high cattle TB incidence. Triplet locations are provided in ref [9]. All trial areas within each triplet were surveyed for badger activity before being randomly assigned to treatments such that each treatment – proactive culling, no culling, or localised “reactive” culling (conducted in response to specific TB breakdowns in cattle herds) – was replicated 10 times, once within each triplet. Badgers were captured in cage traps and despatched by shooting with a pistol; capture protocols took careful account of badger welfare [12,13] and despatch was deemed ‘humane’ by independent audit [14]. Initial culls for each proactive trial area were completed between December 1998 and December 2002. Proactive culls were repeated approximately annually until culling ended in October 2005.

Data on the incidence of confirmed cattle TB breakdowns were downloaded from Defra’s VetNet database, for herds inside RBCT areas and on adjoining land up to 2 km outside RBCT areas. Following ref [11] (which presented analyses of data available in January 2008), we analysed incidence data from two periods. We defined the “during-trial” period as running from the end of the initial proactive cull in each triplet, to exactly one year after completion of the last cull in that triplet, when another annual cull would have been conducted had the proactive treatment been continued. We defined the “post-trial period” as running from the end of the during-trial period up to the most recent data download (7th July 2009). To examine temporal trends, we further divided the during-trial period into intervals between successive culls (e.g. third to fourth cull), and divided the post-trial period into six-month intervals.

As in previously published analyses [8,9,11,15], we used log-linear Poisson regression to compare the numbers of confirmed

breakdowns recorded in and around trial areas subjected to the proactive and no-culling treatments. The regression models adjusted for triplet, the log of the number of baseline herds at risk, and the log of the number of confirmed breakdowns recorded in a three year period before RBCT culling commenced. Where results were stratified by time, a triplet*time interaction term was also included in the model. We adjusted confidence intervals (CI) and p-values for any extra-Poisson overdispersion by using an adjustment factor (the square root of the model deviance divided by the degrees of freedom) in all cases where its value was greater than 1.

Following examination of effects by six-month interval in the post-trial period, we fitted a linear trend (on a log scale) to the effects inside trial areas, and tested this trend against the null hypothesis of no trend. Additionally, in the adjoining areas, we grouped together the first 18 months of the post-trial period and tested the effect in this time period against the null hypothesis of no effect. We used previously published methods [10,11] to investigate whether the effect of culling varied with distance from the trial area boundary.

As in previously published analyses [10,11], we extrapolated from our results to estimate the size of an idealised circular culling area that would need to be targeted to obtain an overall reduction in the incidence of confirmed breakdowns, with detrimental effects outside the targeted area offset by beneficial effects inside. These extrapolations covered the time period from completion of the first proactive cull until effects were no longer detectable.

We calculated the financial costs and benefits of culling, using estimates of the costs of culling, and the benefits of preventing a breakdown, from ref [16]. The benefits included the prevention of both direct and indirect costs associated with: the loss of slaughtered cattle; movement restrictions; isolation; spread to other herds; as well as cattle testing (of the affected herd until the breakdown is cleared, of contiguous herds and of traced cattle linked to the affected herd) [16]. Although updated estimates of the costs of cage trapping have been published recently [17], in the absence of updated costs for other culling methods, or for experiencing a breakdown, we have used the 2005 estimates to ensure comparability. We based calculations on an idealised circular culling area large enough to give an overall beneficial effect over the period from completion of the first proactive cull until effects were no longer detectable. As in previous analyses [10], we assumed that only 75% of targeted land was accessible, reducing the cost of culling.

Results

Inside Culling Areas

Across the entire post-trial period, the incidence of confirmed breakdowns inside proactive culling areas was 37.6% lower (95% CI: 24.6% to 48.4% lower) than that inside no-culling areas (Table 1). Dividing the post-trial period into six-month intervals revealed a significant ($p = 0.038$) linear trend (on a log scale) over time, with the beneficial effect declining by 14.3% with each six-month interval (Figure 1). By months 31–36, no beneficial effect was detectable (Table 1). For the 30-month period when effects were detectable, proactive culling was associated with a 42.0% reduction (95% CI: 24.1–55.6% reduction) in the incidence of cattle TB.

Across the combined during- and post-trial period, the incidence of confirmed breakdowns was 28.7% lower (95% CI: 20.8% to 35.8% lower) in proactive areas than in no-culling areas. For the period comprising the during-trial period and the first 30 months of the post-trial period (when beneficial effects were

Table 1. Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns inside trial areas.

	Triplet-years	Proactive effect	Overdispersion*		P-value for linear trend over time	
		Estimate (95% CI)	p-value	factor	p-value	
<i>During-trial period</i>						
1 st to 2 nd cull	12.6	−3.5% (−30.6% to 34.1%)	0.83			
2 nd to 3 rd cull	13.2	−12.8% (−36.6% to 20.1%)	0.40			
3 rd to 4 th cull	8.4	−39.4% (−57.6% to −13.4%)	0.006			
After 4 th cull to end	21.5	−31.5% (−46.8%to −11.9%)	0.003			
<i>Entire during-trial period</i>	55.7	−23.2% (−32.7% to −12.4%)	<0.001	0.67	0.87	0.15
<i>Post-trial period</i>						
Months 1–6	5.0	−52.7% (−71.8% to −20.8%)	0.004			
Months 7–12	5.0	−41.1% (−64.0% to −3.8%)	0.034			
Months 13–18	5.0	−49.4% (−67.9% to −20.4%)	0.003			
Months 19–24	5.0	−27.8% (−52.4% to 9.4%)	0.094			
Months 25–30	5.0	−35.0% (−59.5% to 4.3%)	0.074			
Months 31–36	3.9	9.9% (−36.7% to 90.7%)	0.74			
Months 37–42	0.4	−†				
<i>Entire post-trial period</i>	29.3	−37.6% (−48.4% to −24.6%)	<0.001	1.08	0.32	0.038
During- and post-trial periods combined	85.0	−28.7% (−35.8% to −20.8%)	<0.001	0.72	0.85	

Analyses adjust for triplet, baseline herds, and historic cattle TB incidence (over three years) and include the entire during- and post-trial periods.

*The analysis dividing both during- and post-trial periods into shorter intervals has overdispersion factor 1.21, $p = 0.003$; †Insufficient breakdowns to calculate estimates. doi:10.1371/journal.pone.0009090.t001

detectable), there was no significant linear effect of distance from the trial area boundary on the magnitude of the beneficial effect (Table 2).

Adjoining Lands

Across the entire post-trial period, the incidence of confirmed breakdowns on lands ≤ 2 km outside proactive culling areas was

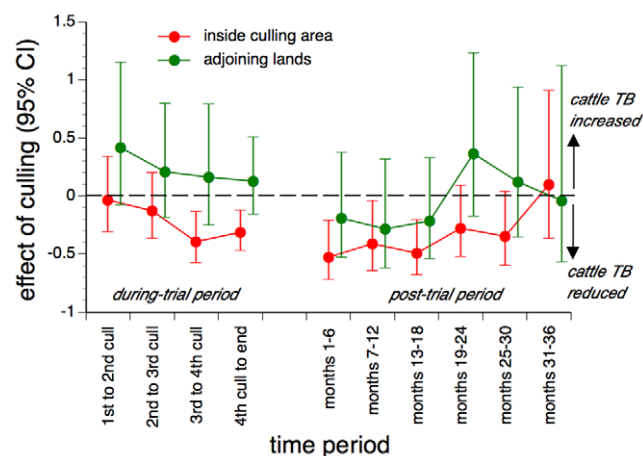


Figure 1. Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns. Estimates are presented for herds inside trial areas as well as those on adjoining lands ≤ 2 km outside trial area boundaries. The estimated effects of proactive culling are stratified by time periods defined by the cull dates in the during-trial period, and by 6-month intervals from 1 year after the last proactive cull (the post-trial period). doi:10.1371/journal.pone.0009090.g001

comparable (5.6% lower, 95% CI: 31.4% lower to 30.0% higher, $p = 0.73$) with that ≤ 2 km outside no-culling areas (Table 3). Dividing the post-trial period into six-month intervals revealed that the effects of culling were estimated to be beneficial for the first 18 months of the post-trial period but never significantly so (20.4% lower in the first 18 months, 95% CI: 41.3% lower to 8.0% higher, $p = 0.19$) (Table 3). For the 30-month period when effects were detectable inside trial areas, the incidence of confirmed breakdowns on lands ≤ 2 km outside proactive culling areas was comparable (6.0% lower, 95% CI: 29.7% lower to 25.7% higher, $p = 0.68$) with that ≤ 2 km outside no-culling areas.

Across the entire combined during- and post-trial period, the incidence of confirmed breakdowns on lands ≤ 2 km outside proactively culled areas was comparable (11.7% higher, 95% CI: 12.9% lower to 43.2% higher, $p = 0.38$) with that ≤ 2 km outside no-culling areas. For the period comprising the during-trial period and the first 30 months of the post-trial period (when beneficial effects were detectable inside trial areas), there was no significant linear effect of distance from the trial area boundary on the magnitude of the effect (Table 2).

Extrapolation to Culling Areas of Different Sizes

Extrapolations to culling areas of different sizes assume an idealised circular area to be targeted by culling, surrounded by a 2 km-wide annulus of adjoining land. Since there was no significant trend in the effects by distance from the trial area boundary (Table 2), extrapolations assumed that effects were consistent throughout the affected areas. Extrapolations were based on effects over the entire during-trial period, plus the 30 months of the post-trial period when effects were still detectable. Within these assumptions, the overall average effect of proactive culling was predicted to lead to a net reduction in the overall incidence of

Table 2. Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns at varying distances inside and outside trial area boundaries, over the period from the initial culls to the end of first 30 months of the post-trial period.

	Proactive effect		Overdispersion		P-value for linear trend
	Estimate (95% CI)	p-value	factor	p-value	
<i>Inside trial areas</i>					
0–1 km inside	–20.4%(–35.4% to –2.1%)	0.031	1.39	<0.001	0.18
1–2 km inside	–25.9%(–42.8% to –4.1%)	0.022			
2–3 km inside	–31.3%(–50.3% to –5.1%)	0.023			
3–4 km inside	–22.2%(–52.8% to 28.0%)	0.32			
4–5 km inside	–46.0% (–85.7 to 103.6%)	0.36			
Entire trial area	–28.7%(–35.8% to –20.7%)	<0.001	0.86	0.63	
<i>Adjoining lands ≤2 km outside trial areas</i>					
0–0.5 km outside	–18.0%(–38.0% to 8.5%)	0.16	1.19	0.017	0.61
0.5–1 km outside	35.8% (2.7% to 79.5%)	0.032			
1–1.5 km outside	–2.9%(–28.1% to 31.1%)	0.85			
1.5–2 km outside	14.3%(–18.7% to 60.7%)	0.44			
Entire area of adjoining land	11.7%(–13.0% to 43.4%)	0.39	1.84	0.001	

Analyses adjust for triplet, baseline herds, and historic cattle TB incidence (over three years).
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confirmed herd breakdowns when targeted at circular areas larger than 17 km² (Figure 2). However, the 95% CI for the average effect across the entire affected area only excluded net increases in the overall incidence of confirmed herd breakdowns for culling targeted at circular areas greater than 141 km² (Figure 2).

Financial Costs and Benefits

Illustrative calculations of the costs and benefits of culling covered the five-year during-trial period of annual culls (from the completion of the initial cull to one year after the fifth cull) plus the subsequent 2.5 years during which culling effects were detectable.

Table 3. Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns on lands ≤2 km outside trial areas.

	Triplet-years	Proactive effect	Overdispersion*		P-value for linear trend over time	
		Estimate (95% CI)	p-value	factor	p-value	
<i>During-trial period</i>						
1 st to 2 nd cull	12.6	43.1%(−5.6% to 116.8%)	0.091			
2 nd to 3 rd cull	13.2	22.8%(−16.9% to 81.7%)	0.30			
3 rd to 4 th cull	8.4	17.8%(−23.4% to 81.1%)	0.45			
After 4 th cull to end	21.5	14.7%(−13.8% to 52.6%)	0.35			
Entire during-trial period	55.7	24.5%(−0.6% to 56.0%)	0.057	1.26	0.13	0.077
<i>Post-trial period</i>						
Months 1–6	5.0	−17.5%(−51.2% to 39.5%)	0.47			
Months 7–12	5.0	−26.9%(−60.0% to 33.5%)	0.31			
Months 13–18	5.0	−19.5%(−51.9% to 34.8%)	0.41			
Months 19–24	5.0	37.9%(−15.5% to 125.2%)	0.20			
Months 25–30	5.0	14.1%(−33.5% to 95.5%)	0.63			
Months 31–36	3.9	−2.1%(−55.2% to 113.8%)	0.96			
Months 37–42	0.4	−†				
Entire post-trial period	29.3	−5.6%(−31.4% to 30.0%)	0.73	1.51	0.025	0.17
During- and post-trial periods combined	85.0	11.7%(−12.9% to 43.2%)	0.38	1.83	0.001	

Analyses adjust for triplet, baseline herds, and historic cattle TB incidence (over three years) and include the entire during- and post-trial periods.

*The analysis dividing both during- and post-trial periods into shorter intervals has overdispersion factor 1.14, p = 0.030; †Insufficient breakdowns to calculate estimates.
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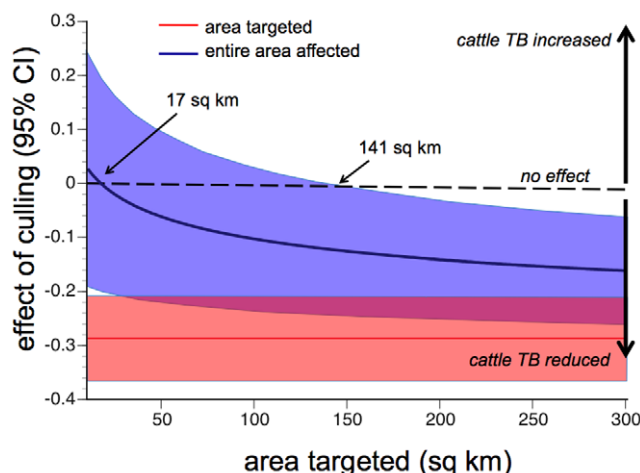


Figure 2. Extrapolation of overall effects to culling areas of different sizes. The blue area shows the 95% confidence interval for the overall impact (combining the impact inside the targeted area with that seen ≤ 2 km² outside) of different sized circular culling areas. The red area shows the impact inside the targeted area only. The estimated overall effect is of increased incidence for areas smaller than 17 km², moving to a decreased incidence when areas larger than 17 km² are targeted. The effect of decreased overall incidence is statistically significant for areas larger than 141 km². doi:10.1371/journal.pone.0009090.g002

Over these 7.5 years, in the absence of any culling, an idealised circular area of 150 km², with a herd density of 1.25/km² and a background incidence of 0.08 breakdowns/herd/year, would be expected to experience 112.5 herd breakdowns. Over the same period, adjoining lands (99 km² falling ≤ 2 km outside the circular area) would experience 74.3 breakdowns, giving a combined total of 186.8. During a five-year culling period, annual proactive culling in the circular area would be expected to prevent 23.2% of 75 breakdowns inside the culled area (17.4 breakdowns prevented), while increasing the number of breakdowns on adjoining land by 24.5% (prompting 12.1 additional breakdowns), giving an overall total of 5.3 breakdowns prevented. In the 2.5 years following culling, the number of breakdowns inside the culled area would be reduced by 42.0% (15.8 breakdowns prevented), and the number on adjoining lands would be reduced by 6.0% (1.5 breakdowns prevented), giving an overall total of 17.3 breakdowns prevented. Hence, the total impact of culling such an idealised area would be to prevent 22.6 breakdowns over 7.5 years. This constitutes a saving of £610,200 at £27,000/breakdown [16]. For comparison, the cost of conducting five annual culls over a 150 km² area, 75% of which was accessible for culling, is estimated as £2.14 million for cage trapping (as undertaken in the RBCT) at £3,800/km²/year, or £1.35 million for snaring or gassing at roughly £2,400/km²/year [16].

Discussion

The results presented here show the duration of reductions in cattle TB incidence associated with widespread badger culling. Beneficial effects inside culled areas were greatest shortly after culling ended, but then declined over time and were no longer detectable four years after the last annual cull (i.e. three years into the post-trial period). On adjoining lands, the effects of culling were estimated to be beneficial only for the first 18 months of the post-trial period but never significantly so.

Although there have been a number of assessments of the effects of badger culling on cattle TB, our study provides the only

experimentally-derived estimate of the duration of effects following the cessation of culling. There has been one other large-scale replicated trial of the effects of badger culling on cattle TB incidence, albeit without the randomised allocation of treatments, or the no-culling control [18]. This study, conducted in the Republic of Ireland and known as the Four Areas Trial, found reductions in cattle TB incidence ranging from 51% to 68% over a five-year culling period [18]. One explanation for the larger beneficial effect of ongoing culling observed in the Four Areas Trial is that greater reductions in badger density may have been achieved, because (i) land occupier compliance was higher; (ii) the use of snares, rather than cage traps, probably allowed a higher proportion of badgers to be captured; and (iii) the culling areas were selected to have geographical barriers such as coastline and rivers which would impede badger recolonisation. However, since culling is still ongoing in the Four Areas, that study provides no data on the duration of impacts post-culling which can be compared with the results presented here. Similarly, Kelly *et al.* [19] studied the long-term effects of badger culling on cattle TB using 16 years of observational data, but badger culling was ongoing throughout (with some periods having more intensive culling than others).

In the absence of data on badger populations during the post-trial period, we cannot be certain of the ecological and epidemiological mechanisms underlying the changes in cattle TB risks that we documented in and around former RBCT culling areas. However, we suspect that these changes reflect recovery of badger numbers and spatial organization following the cessation of culling. Proactive culling markedly reduced local densities of badgers [20], which would be expected to reduce the overall risk of cattle coming into contact with badgers. However, culling also prompted expansions of badger ranging behaviour [21,22], increasing the number of herds that each badger could potentially contact. Moreover, culling increased the prevalence of *M. bovis* infection among badgers [23,24]; this, combined with badgers' expanded ranging, would increase the probability of badger-to-cattle transmission, undermining the beneficial effects of reduced badger density. In another study, cessation of culling prompted a contraction of badger ranging within about two years, but recovery of badger numbers took around 10 years [25]. We previously suggested [11] that the marked reductions in cattle TB incidence observed immediately after the cessation of culling might reflect contraction of badger home ranges (and consequently reduced contact with cattle) prior to substantial recovery of badger numbers. We further speculate that the subsequent decline and disappearance of these beneficial effects may reflect increasing badger numbers, and consequently increased badger-cattle contact. While it is impossible to determine whether the system has now returned to equilibrium, in other studies badger numbers have taken five [26] to ten [25,27] years to recover from culls, suggesting that growth of the badger populations in RBCT proactive areas may continue for several more years. As the prevalence of *M. bovis* infection in badgers was found to rise on successive culls [23], it is possible that the prevalence in badgers might still be elevated in RBCT areas (although no data are available to test this hypothesis). Were this the case, however, continued growth of the badger populations might be associated with future increases in the risk of TB transmission to cattle herds in areas proactively culled during the RBCT. Continued surveillance of cattle herds will allow characterisation of any further changes in cattle TB incidence, while studies of badger population density, spatial organization, and *M. bovis* infection prevalence could provide ecological and epidemiological insights into the long-term impacts of culling, and its cessation, on bovine TB dynamics.

It is important to note that the effects described here relate only to culling as conducted in the RBCT, *i.e.* deployment of cage traps by highly trained staff in coordinated, large-scale, simultaneous operations, repeated annually for five years and then halted. As described elsewhere, culling-induced changes in badger numbers and movement patterns mean that culling which is small-scale, patchy, short-term or asynchronous is very unlikely to provide comparable reductions in the incidence of cattle TB and could well prompt increases [8,9,10,15,21,23,24]. Other culling methods, such as snares or gassing, might be expected to remove a higher proportion of local badger populations than did cage traps (albeit with a likely cost in terms of badger welfare). However, since there is both ecological [20] and genetic [22] evidence that badger culling prompts substantial immigration from surrounding lands, improvements in culling efficiency might not result in proportional reductions in badger density, and would not therefore be expected to greatly improve the beneficial effects of culling. In principle, such immigration could be limited by culling within geographical features which present barriers to badger movement (as in the areas selected for culling in Ireland's Four Areas Trial [18]). However, such geographical barriers are sparse in TB-affected areas of Britain [e.g., 28]. Detailed consideration of other potential forms of badger culling [10] suggests that no practicable methods would be likely to yield benefits markedly greater than those achieved in the RBCT.

Our results suggest that culling would need to be targeted at circular areas larger than 141 km² for long-term benefits to be realised. Because the relative benefits improve only slowly with increasing area culled (Figure 2), even larger areas would need to be targeted to be confident of benefits substantially greater than break-even. For example, to be confident of achieving at least a 10% reduction in the overall incidence of cattle TB would require targeting culling at circular areas ≥ 568 km². These extrapolated figures are somewhat larger than those published most recently, because earlier extrapolations assumed that the benefits of culling increased at greater distances inside the culling area boundary [11]. Since no such trend is detectable in this updated dataset, it was excluded from the calculations presented here. All such extrapolations are illustrative: in reality, deviations from perfectly circular culling areas would increase edge effects and reduce overall benefits, while positioning of culling areas close to cattle-free areas or geographic barriers to badger movement might potentially reduce edge effects and increase net benefits [10]. Nevertheless, such extrapolations give a rough indication of the minimum areas within which culling would need to be conducted for benefits to be realised.

These updated findings also allow an assessment of the financial costs and benefits of badger culling as a tool to control cattle TB. The overall number of breakdowns estimated to be preventable by proactive culling is fairly modest in comparison with background TB incidence (e.g. 22.6 breakdowns prevented over 7.5 years in an area that would otherwise experience roughly 187 breakdowns), and the consequent financial savings much too low to offset the costs of culling using cage traps, snares, or gassing. Defra estimated that the costs of culling would be substantially lower if implemented by licencing of farmers (roughly £1,000/km²/year [16], hence £562,500 for the idealised five-year 150 km² area described above; note that the Welsh Assembly Government recently published updated cost estimates of £4,200/km²/year for government-delivered cage trapping and £1,500/km²/year for farmer-delivered culling [17]). However, this assumed that farmers would conduct the culling themselves (and so included only minimal capital costs) and excluded the costs of training farmers or coordinating their efforts [16]. In the absence of such

training and coordination, licenced culling would almost certainly be patchy, asynchronous, unsustainable and uncoordinated, circumstances highly likely to prompt increases, rather than reductions, in the incidence of cattle TB [10,15,23,24]. Hence, although the total cost of licenced culling is slightly lower than the potential benefits projected from RBCT results (using 2005 cost estimates [16]), it is extremely unlikely that such benefits could in fact be realised by this culling method. The costs of conducting badger culls thus substantially exceed the long-term financial benefits likely to be achieved.

Our findings are broadly consistent with those of a recent analysis [29] which assessed the potential financial outcomes of badger culling by combining a transmission model (incorporating aspects of badger ecology such as post-cull disruption of badger social organization, as well as farm management such as cattle movement) with data on costs and benefits. In this model, cage-trapping of badgers (assumed to remove 70% of badgers), produced a net economic loss in all simulations, with these losses being greater than those associated with the other culling options considered (shooting free-ranging badgers, snaring and gassing). The authors concluded “*Model results strongly indicate that although, if perturbation [of badger social groups] were restricted, extensive badger culling could reduce rates in cattle, overall an economic loss would be more likely than a benefit.*”

Predicting the financial implications of continuing (rather than halting) annual proactive culls is speculative. However, we can estimate the financial costs and benefits to be incurred annually in and around the idealised circular area of 150 km² (with a herd density of 1.25/km² and a background incidence of 0.08 breakdowns/herd/year) based on the impacts of culling estimated between the fourth proactive cull and the end of the during-trial period (the latest estimates available while the proactive culling treatment was ongoing, Tables 1 and 3). On this basis, each year of annual proactive culling in the circular area would be expected to prevent 31.5% of 15 breakdowns inside the culled area (4.7 breakdowns prevented), while increasing the number of breakdowns on adjoining land by 14.7% (prompting 1.4 additional breakdowns), giving an overall total of 3.3 breakdowns prevented on average. This constitutes an annual saving of £89,100 at £27,000/breakdown [16]. For comparison, the cost of conducting an annual culls over a 150 km² area, 75% of which was accessible for culling, is estimated as £427,500 for cage trapping (as undertaken in the RBCT) at £3,800/km²/year, or approximately £270,000 for snaring or gassing at roughly £2,400/km²/year [16]. Clearly, continuing to cull would be relatively costly were the benefits of ongoing annual culling to continue at the levels observed following the fourth and subsequent proactive culls in the RBCT.

Our findings have important implications for the development of cattle TB control policies throughout the British Isles. They show that, although widespread badger culling can achieve overall reductions in the incidence of cattle TB, these benefits are not sustained in the long term once culling is halted. Moreover, the financial costs of conducting the culling substantially exceed the overall benefits accrued. In the absence of other practicable culling methods likely to yield greater benefits, our findings indicate that, on the basis of cost-effectiveness, badger culling is unlikely to contribute to the control of cattle TB in Britain.

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Conceived and designed the experiments: HEJ RW CAD. Analyzed the data: HEJ CAD. Wrote the paper: HEJ RW CAD. Interpreted the results. Interpreted the results: HEJ RW CAD.

Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings

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A novel influenza A (H1N1) virus has spread rapidly across the globe. Judging its pandemic potential is difficult with limited data, but nevertheless essential to inform appropriate health responses. By analyzing the outbreak in Mexico, early data on international spread, and viral genetic diversity, we make an early assessment of transmissibility and severity. Our estimates suggest that 23,000 (range 6000 to 32,000) individuals had been infected in Mexico by late April, giving an estimated case fatality ratio (CFR) of 0.4% (range: 0.3 to 1.8%) based on confirmed and suspected deaths reported to that time. In a community outbreak in the small community of La Gloria, Veracruz, no deaths were attributed to infection, giving an upper 95% bound on CFR of 0.6%. Thus, although substantial uncertainty remains, clinical severity appears less than that seen in the 1918 influenza pandemic but comparable with that seen in the 1957 pandemic. Clinical attack rates in children in La Gloria were twice that in adults (<15 years of age: 61%; ≥15 years: 29%). Three different epidemiological analyses gave basic reproduction number (R_0) estimates in the range of 1.4 to 1.6, whereas a genetic analysis gave a central estimate of 1.2. This range of values is consistent with 14 to 73 generations of human-to-human transmission having occurred in Mexico to late April. Transmissibility is therefore substantially higher than that of seasonal flu, and comparable with lower estimates of R_0 obtained from previous influenza pandemics.

On 29 April 2009, the World Health Organization (WHO) announced that the rapid global spread of a strain of influenza A (H1N1) virus detected in the previous week warranted moving the global pandemic alert level to phase 5 (www.who.int/csr/disease/swineflu/). Phase 5 indicates sustained human-to-human transmission of a novel influenza strain of animal origin in one WHO region of the world, and exported cases detected in other regions. In this outbreak, the earliest affected country may have been Mexico, with many cases in other nations associated with

travels from that country. There are uncertainties about all aspects of this outbreak, including the virulence, transmissibility, and origin of the virus, and this in turn results in uncertainty in judging the pandemic potential of the virus and when reactive public health responses, such as recommendations to stay at home or to close schools, should be implemented in individual countries. Here we report findings of key early investigations into the outbreak that could aid such policy decisions.

The presence of fatalities [29 confirmed plus 88 suspected deaths in Mexico as of 4 May 2009 (1), 1 confirmed in the United States as of 5 May 2009 (2)] is not necessarily indicative of the virulence of the infection. The interpretation of these statistics depends on the total number of infections, including those with mild infection or who are asymptomatic, which is currently unknown, given the absence of a specific serological test for the new H1N1 influenza strain and associated population-level screening. As of 4 May 2009, 11,356 suspected and 822 laboratory-confirmed cases have been reported in Mexico (1), but these may represent an underestimate of true case numbers as surveillance has understandably focused on severe cases. Furthermore, severe cases in older individuals will be more difficult to identify because of the higher rate of respiratory illness in

those over 60 years of age (3), and this could result in an underestimate of overall morbidity. Right censoring of mortality data, which occurs when additional deaths subsequently arise among cases already included in surveillance data, can also bias estimates of the true case fatality ratio (4). Finally, suspected deaths may not all have been caused by infection with the novel virus. These uncertainties necessarily affect any estimate of the case fatality ratio (CFR).

On the basis of international travel patterns, we would expect a proportion of cases of any infection spreading widely in Mexico to be exported by travelers (5). Owing to intense surveillance for influenza-like illness in those returning from Mexico, ascertainment of early cases in newly affected countries was almost certainly more complete and rapid than local surveillance of mild cases in Mexico. Airline passenger flow out of Mexico shows a significant correlation with the frequency of detected confirmed cases worldwide (Spearman correlation coefficient: 0.56, $P = 0.004$) (Fig. 1, A and B). We thus use data on cases among travelers and backcalculation methods to estimate the total number of people infected in Mexico. Key underlying assumptions in this analysis are that population mixing in Mexico is equally likely between Mexican residents and tourists, and tourists and Mexican residents are at equal risk of infection (despite demographic and other differences). If infections are concentrated away from traveler destinations (Fig. 1E presents the spatial distribution, by state, of cases within Mexico by 5 May), the number of people infected in Mexico will be underestimated, and conversely will be overestimated if the epidemic has disproportionately affected geographical zones visited by travelers. Under the assumption that reporting of infections in travelers was complete, we estimated the number of infections that occurred in Mexico by late April from a model of the interval-censored country case counts, which varied between 18,000 and 32,000 (Table 1), depending on the mean duration of stay of tourists assumed, with perhaps the most credible single value (based on journey duration data) being 23,000. An alternative model that assumed at least one case had been confirmed in every country affected by late April gave lower estimates of the number infected in Mexico, in the range of 6000 to 11,000. However, this model may be viewed as a worst case (from the perspective of resulting CFR estimates), and it fitted the observed number of exported cases in key countries (such as the United States and Canada) substantially worse than did the first model. We used 30 April 2009 as the cut-off date for the data analyzed, but the case data analyzed are subject to delays (clinical onset, testing, and reporting) of up to 1 week, so these estimates may be more representative of infections up to 23 April. The epidemic has subsequently spread further, although the impact of the nonpharmaceutical interventions introduced in Mexico is not yet known.

On the basis of the 9 confirmed and 92 suspected deaths that were reported by 30 April 2009

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(6) and assuming similar times from infection to confirmation and from infection to death, we estimated CFRs in the range of 0.3 to 0.6% from the interval-censored case count model, based on confirmed and suspected deaths combined, or 0.03 to 0.05% for confirmed deaths only. Using the alternative, more pessimistic, country presence/absence model, we estimated CFRs of 0.9 to 1.8% based on suspected and confirmed deaths, and 0.08 to 0.16% from the confirmed deaths alone. These estimates have already changed somewhat as a result of data available after 30 April, but we deliberately report the earlier analysis because it formed part of the evidence base used by WHO to move to phase 5.

Another source of information on severity comes from the large outbreak of respiratory disease seen in the small, isolated community of La Gloria in Veracruz province, one case of which has been confirmed to have been caused by the novel H1N1 strain. It is possible that other viruses were circulating at the same time as the outbreak, but the overall attack rate is substantially larger than would be expected for a seasonal influenza outbreak. No fatalities among 616 cases have been attributed to infection during the full period of surveillance of that outbreak (Fig. 3A), giving a 95% confidence interval (CI) of 0 to 0.60%.

Data on the magnitude of the current outbreak in Mexico can also be used to estimate the transmissibility of the virus if the start date of the outbreak is known or can be estimated. Epidemiological investigations into the emergence of the virus in Mexico have focused on the La Gloria outbreak, where the first case in that outbreak is thought to have occurred around 15 February 2009 (Fig. 3A).

An alternative approach to estimating the start date of the outbreak is to look at the diversity in the genetic sequences of viral samples collected from confirmed cases, assuming that diversity accumulates according to a molecular clock model. Twenty-three complete publicly available hemagglutinin (HA) gene sequences from cases not linked in epidemiological clusters were analyzed with a Bayesian coalescent method that assumes exponential growth of the viral population (7). This yielded an estimate of the time of most recent common ancestor (TMRCA) of 12 January 2009 [95% credible interval (CrI): 3 November 2008 to 2 March 2009]. The genetic model also gave an estimate of the doubling time of the epidemic of 10 days (95% CrI: 4.5 to 37.5 days) (Fig. 2). Assuming exponential growth, the TMRCA is a reasonable estimate of the start of the outbreak, although it is formally an upper bound due to incomplete sampling of the epidemic and the effects of the exponential model prior to distribution. These findings from a population genetic analysis are consistent with the epidemiological investigation of both the start and magnitude of the current epidemic in Mexico. Figure 2 also shows a preliminary version of this analysis based on the first

11 sequences, which gave similar estimates highlighting the power of these methods. [See (8) for further sensitivity analysis and methods.]

The reproduction number, defined as the number of cases one case generates on average over the course of their infectious period, is a key measure of transmissibility and can be estimated in a number of ways from the data currently available.

First, by assuming exponential growth, the growth rate of the epidemic (r) can be inferred from estimates of the current cumulative number of infections (Y_t) and estimated start date and size for the outbreak (t_0 and Y_0 , respectively). The basic reproduction number (R_0) can be estimated from the exponential growth rate if one also assumes that the generation time distribution for

the new H1N1 strain is similar to that of other strains of seasonal and pandemic viruses (9, 10) [Table 1 and (8)]. Using the date of 15 February as the first case of the La Gloria outbreak (8) gives reproduction number estimates of between 1.31 and 1.42, depending on which variant of the geographical backcalculation model is used. Extending a more sophisticated Bayesian estimation method (11) that allows for stochastic variability intrinsic to epidemic dynamics and parameter uncertainty gave similar but slightly higher estimates for R_0 with wider ranges: posterior median = 1.40; 95% CrI: 1.15 to 1.90 (Fig. 1C).

Second, by assuming a prior distribution on the generation time distribution informed by previous estimates of influenza, the Bayesian co-

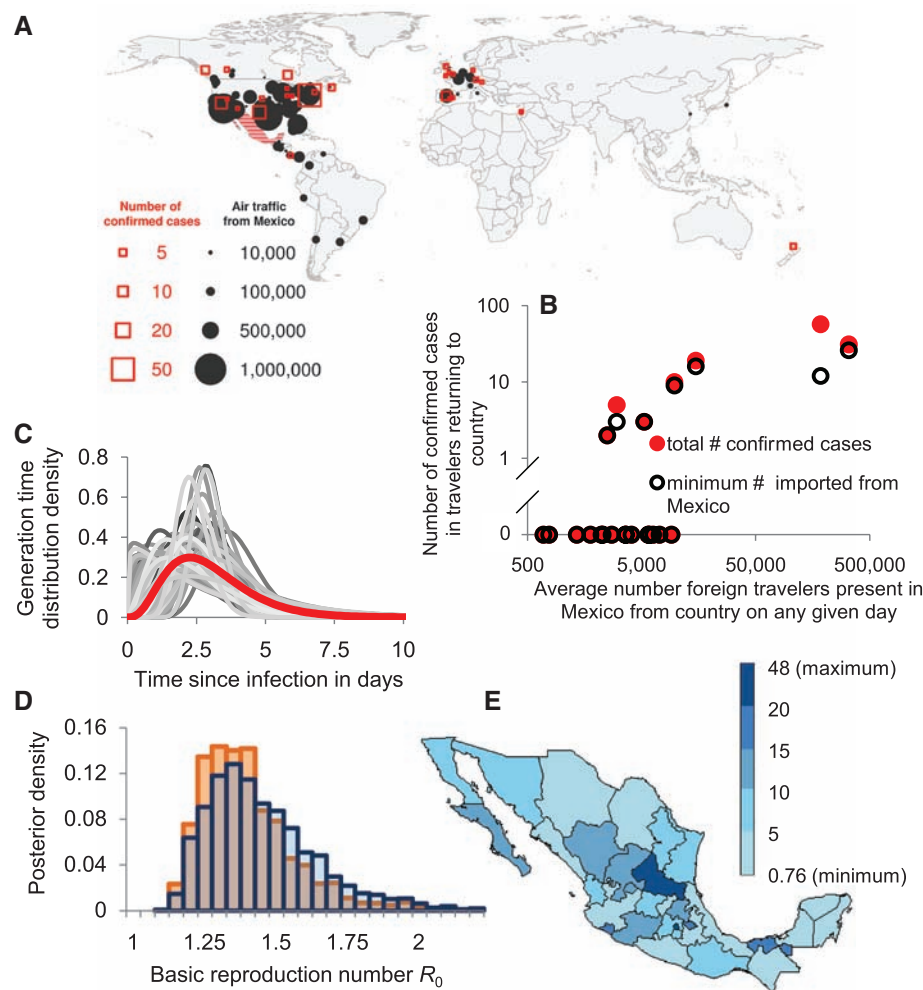


Fig. 1. (A) The number of passengers flying out of Mexico by actual destination and the number of confirmed cases as reported on 30 April 2009. (B) The number of cases exported to country j as reported on 30 April 2009 as a function of the estimated average number of foreign travelers in Mexico from country j on any given day in March or April. Black circles: minimal number based on one exposure per epidemiological cluster; filled red circles, total number of confirmed cases. (C) Mean assumed generation time distribution (red) and 100 illustrative draws from the prior distribution, and (D) corresponding posterior distribution of R_0 estimates for a stochastic model of an epidemic within Mexico with travelers infected at a rate proportional to the estimated density of travelers per local resident. The two bar charts correspond to a 7-day delay between infection and confirmation (blue) and no delay (orange) in cases among travelers. (E) Number of acute respiratory infection cases per 100,000 inhabitants by state as reported on 5 May 2009 (1), demonstrating spatial distribution of disease within Mexico.

lescent population genetic analysis yielded a second set of estimates for R_0 : posterior median = 1.22; 95% CrI: 1.05 to 1.60 (Fig. 2C).

Third, R_0 can also be estimated from analysis of the dynamics of the epidemic within defined settings. Detailed data collected by the Mexican authorities investigating the La Gloria outbreak indicate that 616 individuals from a resident population of 1575 had acute respiratory infection between 15 February and 14 April 2009 (Fig. 3A). Data on the age distribution of cases and the dates of disease onset were used in our analysis. Figure 3B shows that the clinical attack rate varied markedly as a function of age, with 61% of individuals under 15 years old affected, dropping to 29% of people over that age. The corresponding relative

risk is 2.13, with a 95% CI of 1.89 to 2.39. Based on all confirmed cases in Mexico as reported on 5 May 2009 (1), the corresponding relative risk is 1.52 (95% CI: 1.33 to 1.73). The overall community attack rates seen in La Gloria are comparable to (or higher than) those seen in previous pandemics (12).

Fitting alternative epidemic models to the La Gloria data (8) demonstrated that a model with heterogeneous mixing by age plus age-dependent susceptibility to infection was required to adequately fit the data with plausible parameter estimates. The resulting maximum-likelihood estimate of R_0 was 1.58 with a 95% CI of 1.34 to 2.04 (Table 2). This analysis also provided the only independent estimate of the mean generation time, T_g (1.91 days; 95% CI: 1.30 to 2.71 days) (Table 2), shorter than earlier estimates for influenza (9, 10), though not significantly so. It is biologically plausible that R_0 and T_g could be correlated, because both are linked to the underlying replicative fitness of the virus. More data are needed. Owing to parameter identifiability issues, it was not possible to estimate age-dependent infectiousness, as well as age-dependent mixing, from these data. Although these estimates are informative, it should be emphasized that some uncertainties remain regarding the denominator population and that a range of other models may fit the data as well as the model choice shown here. Household data would be particularly useful in reducing remaining uncertainty.

Fourth, the time-dependent reproduction number (R_t) can be estimated from the time series of

reported disease onsets among confirmed cases in Mexico (Fig. 4). These data are subject to much uncertainty because of marked changes in surveillance over the reporting interval, plus the non-specificity of symptoms that are similar to existing and perhaps simultaneously circulating strains of influenza. However, we developed methods for analyzing such data (8) that account for substantial underreporting, with a change in the underreporting rate from 17 April when surveillance within Mexico was intensified. The average value of R_t estimated for Mexico up until the end of April was 1.37 (95% CrI: 1.24 to 1.59) for a model with Poisson case counts, and 1.47 (95% CrI: 1.21 to 1.88) for a perhaps more plausible negative binomial model allowing daily case counts to be overdispersed (8).

Given estimates of R_0 and the current epidemic size x , we can estimate the number of generations N_t of transmission of the virus among humans that is necessary to explain the current epidemic. Assuming a simple branching process with reproduction number R_0 , the mean number of generations of transmission is given by $N_t = \ln(x/x_0)/\ln(R_0)$, assuming the epidemic was started by x_0 humans being infected from animal sources. Assuming $x_0 = 1$ gives estimates of N_t between 14 and 73. But even if we assume that 5% of cases were infected directly from animal sources, we obtain an estimated 5 to 22 generations of transmission, indicating sustained human-to-human transmission in Mexico.

All of the R_0 estimates are comparable with, but perhaps on the low end of, R_0 estimates

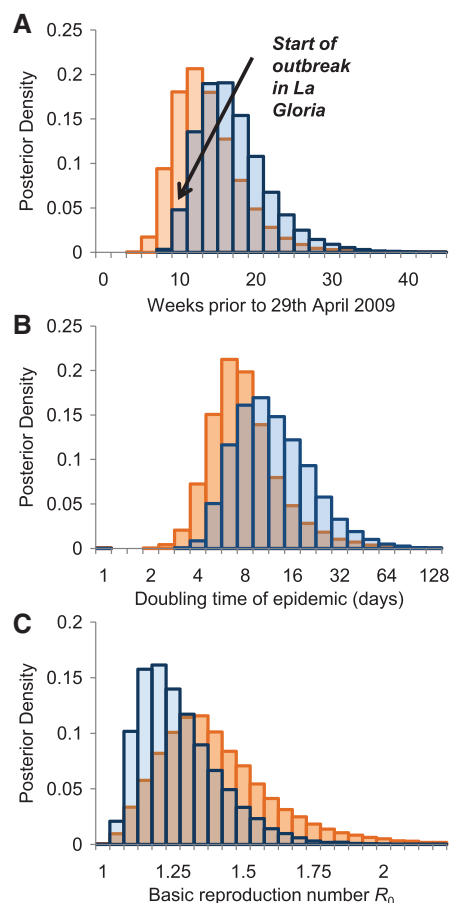


Fig. 2. (A) Starting from publicly available HA viral sequences, a posterior distribution of the estimated TMRCA was derived using a Bayesian coalescent model, which assumes exponential population growth (coded in BEAST 1.4), with the date of the first known human case highlighted. Details of the BEAST analysis and parameter estimates are presented in (8). Posterior distribution of the doubling time of the epidemic (B) and of R_0 (C). The bar charts show the results obtained from the first 11 sequences available on 2 May 2009 (orange) and from an updated analysis with 23 epidemiologically unlinked sequences available on 7 May 2009 (blue). The differences in estimates arise due to some sequences in the smaller sample being from epidemiological clusters, highlighting the importance of careful sampling.

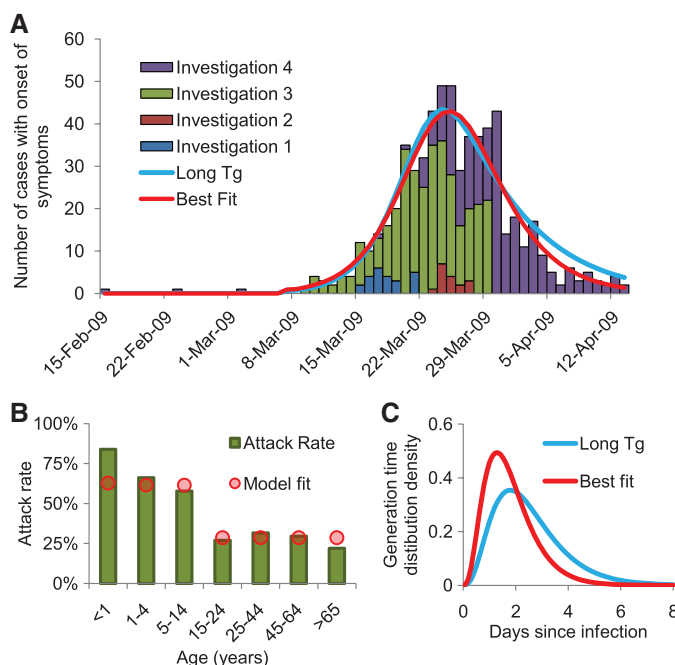


Fig. 3. Results of a detailed investigation into an outbreak in the village of La Gloria. (A) The time series of cases based on repeat rounds of investigation into the outbreak, and the best fit of an age-stratified transmission model (see Table 2 for estimates). The graph also shows the best fit of a model where the generation time is constrained to be consistent with earlier estimates for influenza (2.6 days), which does not fit significantly worse than the unconstrained best fit (see Table 2 legend). (B) Observed (bars) and fitted (using best fit, circles) age-specific attack rates; (C) best fit and constrained estimate of the generation time distribution.

Table 1. Parameter estimates (and 95% confidence intervals) for the cumulative number of influenza A (H1N1) infections in Mexico among Mexican residents by late April 2009, along with corresponding estimates

for the case fatality ratio (CFR), the basic reproduction number, and the exponential growth rate, assuming that infections in travelers occurred in Mexico.

Assumed average duration of stay for visitors arriving by flights	Estimated number of infections among Mexican residents	Case fatality ratio*		Estimated reproduction number $R_0^{\ddagger}$	Estimated exponential growth rate [‡] (doubling time in days [‡])
		Assuming 9 deaths [§]	Assuming 101 deaths [§]		
Based on analysis of interval-censored country counts					
6 days	32,000 (26,000, 39,000)	0.03% (0.02%, 0.03%)	0.32% (0.26%, 0.39%)	1.42	0.123 (5.6)
9 days (23)	23,000 (20,000, 280,00)	0.04% (0.03%, 0.05%)	0.44% (0.37%, 0.52%)	1.40	0.118 (5.9)
12 days	180,00 (150,00, 220,00)	0.05% (0.04%, 0.06%)	0.55% (0.46%, 0.66%)	1.39	0.114 (6.1)
Based on analysis of presence or absence of confirmed disease in countries					
6 days	11,000 (5,000, 27,000)	0.08% (0.03%, 0.20%)	0.90% (0.37%, 2.20%)	1.36	0.106 (6.6)
9 days (23)	7,000 (3,000, 18,000)	0.12% (0.05%, 0.29%)	1.36% (0.56%, 3.30%)	1.33	0.098 (7.0)
12 days	6,000 (2,000, 14,000)	0.16% (0.07%, 0.39%)	1.81% (0.74%, 4.40%)	1.31	0.093 (7.4)

*This is a simple estimate of the CFR (4). The numbers of deaths and the data on cases in countries were both obtained as reported on 30 April. Although censoring is important to consider when estimating the CFR (due to the time interval between case report and death), in these data we have both the time between clinical onset and death within Mexico and the time between clinical onset and case confirmation in other countries to consider. Given uncertainty surrounding both of these distributions, we have made the simplifying assumption that these time intervals are similar and thus that the CFR can be estimated by the deaths within Mexico attributed to the novel influenza A (H1N1) divided by the estimated number of infections among Mexican residents based on data reported up to 30 April. [§]Based on 9 confirmed and 92 suspected deaths (101 total) that were reported by 30 April 2009 (6). [‡]These estimates assume that the estimated cumulative number of influenza A (H1N1) infections in Mexico among Mexican residents relates to the cumulative number of infections up to 23 April 2009 (i.e., 1 week before the data were reported).

Table 2. Epidemiological parameters estimated by fitting an age-stratified mathematical model to the outbreak in the village of La Gloria (Fig. 3). For sensitivity analysis and model selection, we tested several reduced model variants. None fitted significantly worse, but several produced implausible estimates of the generation time. The respective best-fit values are as follows: 1. No asymptomatics and no misreporting, no assortative mixing: $p_{\text{symp}} = 1$, $\theta = 0$, $R_0 = 1.37$, $T_g = 1.39$, and $\rho_{\text{child}} = 2.80$. 2. No asymptomatics and no misreporting: $p_{\text{symp}} = 1$, $R_0 = 1.41$, $T_g = 1.53$ days, $\theta = 0.31$, and $\rho_{\text{child}} = 2.22$. 3. No assortative mixing: same as variant 1. 4. model with long generation time consistent with previous estimates from influenza (also shown in Fig. 3), $T_g = 2.60$ days, $R_0 = 1.97$, $\rho_{\text{child}} = 2.52$, $\theta = 0.51$, and $p_{\text{symp}} = 0.72$. (The symbol “ $\equiv$ ” denotes parameters defined to take fixed values.)

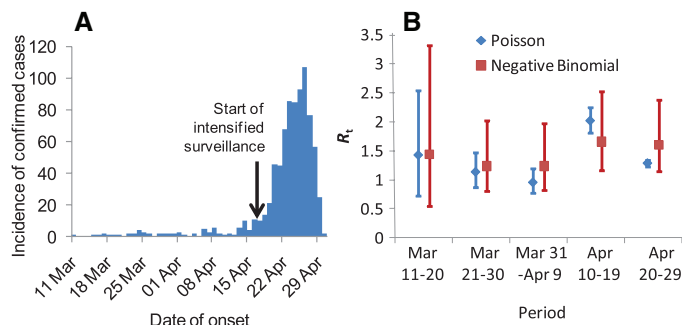
	Best estimate	95% confidence interval	Description
R_0	1.58	1.34–2.04	Basic reproduction number
T_g	1.91	1.30–2.71	Mean generation time (days)
p_{symp}	86%	69–100%	Proportion of cases that are symptomatic and ascertained
ρ_{child}	2.06	1.60–3.31	Susceptibility of children relative to adults
θ	0.50	0.00–0.72	Assortativity of mixing between children and adults (0 = random, 1 = fully assortative)
Assumed value			
f_L	1/3	Assumed	Fraction of the generation time that is latent (uninfectious)
ϕ_{child}	1	Assumed	Infectiousness of children relative to adults

obtained from analysis of previous pandemics [1.4 to 2.0 for 1918, 1957, and 1968 (9, 13–15)]. Overall, our transmissibility estimates are consistent with the lowest values used in earlier detailed computer simulations used to study scenarios in pandemic mitigation (16, 17), indicating that the conclusions regarding control policy effectiveness reached by those analyses could be relevant to the current epidemic. However, the key trade-off remains the balancing of the economic and societal cost of interventions, such as school clo-

sure, against the numbers of lives saved through use of such measures. Where substantial antiviral stockpiles are available, a secondary trade-off is the extent to which large-scale prophylaxis is justified, given the potential risks of high-level resistance developing (18–21). At present, estimates of disease severity are insufficiently robust to allow these trade-offs to be properly evaluated, but that uncertainty should diminish rapidly in coming weeks as more data on severe cases in the United States and other countries become available.

As the situation develops, a key issue is to optimize study designs and surveillance protocols to be most informative in estimating some of these unknown factors, thus potentially informing and refining the public health response. Clearly, detailed investigations of transmission in households and schools will be useful, as would be the consistent collection and dissemination of electronic patient records, which could be used to detect cofactors in the severity of infection. In conclusion, while the emerging data from Mexico and other countries have enabled important insights into the origin, extent, transmissibility, and severity of the unfolding pandemic [including detailed epidemiological analysis of data from the U.S. outbreak recently published (22)], many uncertainties remain and should not be underestimated. The incubation and infectious periods have not yet been reliably ascertained, leaving uncertainty in estimates of the generation time. Much remains to be done to estimate clinical severity of infection, to understand regional variations seen so far (or indeed, whether they exist). As the epidemic spreads further, it is likely that severity will vary from country to country depending on health care resources and the public health measures adopted to mitigate impact. The existence of any cross-immunity (perhaps not mediated via HA-specific antibodies) from past exposure to prior influenza A subtypes is unknown, but the strong age dependence in clinical attack rates seen in La Gloria is intriguing. Cross-immunity would imply that R_0 could be higher in fully susceptible populations than estimated here.

Fig. 4. (A) Time course of the Mexican epidemic with **(B)** the posterior estimates (median and 95% CrI) of the reproduction number over time obtained under Poisson and negative binomial models from the analysis of confirmed cases. The estimate of the negative binomial dispersion parameter k is for a low-to-moderate overdispersion, but this is enough to greatly increase the uncertainty in $R(t)$.



The future evolution of the transmissibility, antigenicity, virulence, and antiviral resistance profile of this or any influenza virus is difficult to predict. It is also unclear whether this strain will displace existing influenza A subtypes from the human population, as occurred in the past three pandemics. The extent to which seasonal damping of transmission in North America and Europe is responsible for the moderate transmissibility seen to date is uncertain; the progress of transmission in the Southern Hemisphere (which is just entering its influenza season) needs to be carefully monitored in the next few months. To reduce all these uncertainties, it is essential that public health agencies around the world continue to collect high-quality epidemiological data in a focused, resource-efficient manner despite the expected increases in case numbers in coming weeks. Epidemiological analysis and modeling are useful tools for guiding such efforts and interpreting the resulting data.

Note added in proof: We cited two sources (1, 6) for confirmed and suspected deaths in Mexico, reported by 4 May 2009 and 30 April 2009, respectively. These sources are not publicly available at present. However, similar reports are publicly available: The Mexican government Web site (24) gives some data on the 5 May situation report (25) documenting 26 confirmed deaths and 114 suspected deaths (77 without samples for analysis), and *Morbidity and Mortality Weekly Report* (26) lists 7 confirmed and 77 suspected deaths posted on 30 April. Since this article appeared online, the number of deaths in Mexico up to 23 April has been determined to be 21, resulting in a revised estimate of the CFR of 0.091% (range: 0.066 to 0.35%) (24).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1176062/DC1
Methods
Figs. S1 to S3
Tables S1 to S12
References
Epidemiological data
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Rapid and Accurate Large-Scale Coestimation of Sequence Alignments and Phylogenetic Trees

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Inferring an accurate evolutionary tree of life requires high-quality alignments of molecular sequence data sets from large numbers of species. However, this task is often difficult, slow, and idiosyncratic, especially when the sequences are highly diverged or include high rates of insertions and deletions (collectively known as indels). We present SATé (simultaneous alignment and tree estimation), an automated method to quickly and accurately estimate both DNA alignments and trees with the maximum likelihood criterion. In our study, it improved tree and alignment accuracy compared to the best two-phase methods currently available for data sets of up to 1000 sequences, showing that coestimation can be both rapid and accurate in phylogenetic studies.

Phylogeny estimation from molecular sequences typically has two phases: An alignment is estimated, and then a tree is produced for the alignment. Alignment methods like MAFFT (1), Probcons (2), Probtrees (3), Prank (4), and Mus-

cle (5) provide more accurate alignments than earlier methods (3, 4, 6), and maximum likelihood (ML) methods of phylogeny estimation [e.g., RAXML (7, 8), GARLI (9), and Phyml (10)] produce more accurate trees for large data sets than other



The effects of annual widespread badger culls on cattle tuberculosis following the cessation of culling

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KEYWORDS

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Cattle

Summary

Background: The effective control of human and livestock diseases is challenging where infection persists in wildlife populations. The Randomised Badger Culling Trial (RBCT) demonstrated that, while it was underway, proactive badger (*Meles meles*) culling reduced bovine tuberculosis (TB) incidence inside culled areas but increased incidence in neighboring areas, suggesting that the costs of such culling might outweigh the benefits.

Objectives and design: The objective of this study was to investigate whether culling impacts persisted more than one year following the cessation of culling (the 'post-trial' period). We compared TB incidence in and around RBCT proactive culling areas with that in and around matched uncultured areas.

Results: During the post-trial period, cattle TB incidence inside culled areas was reduced, to an extent significantly greater ($p = 0.002$) than during culling. In neighboring areas, elevated risks observed during culling were not observed post-trial ($p = 0.038$). However, the post-trial effects were comparable to those observed towards the end of the trial (inside RBCT areas: $p = 0.18$ and neighboring areas: $p = 0.14$).

Conclusions: Although to-date the overall benefits of culling remain modest, they were greater than was apparent during the culling period alone. Continued monitoring will demonstrate how long beneficial effects last, indicating the overall capacity of such culling to reduce cattle TB incidence.

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Introduction

The effective control of human and livestock diseases is challenging where infection persists in wildlife populations.

The Randomised Badger Culling Trial (RBCT)¹ demonstrated that, while it was underway, proactive badger (*Meles meles*) culling reduced bovine tuberculosis (TB) incidence inside culled areas but increased incidence in neighboring areas,^{2–5} suggesting that the costs of such culling might outweigh the benefits.^{1,6,7}

Bovine tuberculosis (TB), a serious disease of cattle, was eliminated from most of Britain by 1960, when the whole

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country was declared attested, using a policy of routine testing of cattle combined with slaughter of affected animals.⁸ However, infection remained in areas of southwest England, where it was linked to infection of local badger (*Meles meles*, Figure 1) populations with *Mycobacterium bovis*, the causative agent of the disease.⁹ Since 1979 incidence in British cattle has increased and the infection has become more geographically widespread.¹⁰ This increase has occurred despite badger culling being an additional component of the British TB control policy from 1973 until the start of the RBCT in 1998.¹⁰

The RBCT¹ was designed to evaluate the effectiveness of two badger culling strategies, by comparing the incidence of cattle TB under three experimental treatments—repeated widespread ('proactive') culling, localized ('reactive') culling, and no culling ('survey-only')—each replicated ten times in large (100 km²) trial areas. Results from the RBCT published at the completion of the proactive strategy, after roughly five years of annual culling,^{2,3} showed that proactive culling reduced cattle TB incidence inside the culled areas. However, incidence was elevated in neighboring uncultured areas (up to 2 km outside the culled areas). The latter effect apparently occurred because culling induced changes in badger behavior,¹¹ which increased the transmission of infection both between badgers,¹² and from badgers to cattle. Localized reactive culling as a once-only event in response to each herd breakdown, likewise was associated with an overall detrimental effect,^{4,5} apparently for similar ecological reasons.¹¹ At the scale and over the period on which RBCT culling was conducted, the detrimental effects (24% increased incidence) observed outside proactive culling areas counteracted the benefits (23% reduced incidence) experienced inside; the relative magnitude of these effects would be expected to vary with the size of the area culled.² The evidence available at the end of the RBCT indicated that a circular culled area of at least 265 km² would be required to provide 95% confidence that the overall impact would, on average, be a net reduction in the incidence of confirmed herd breakdowns.¹

There has been considerable debate about the importance of the detrimental effects associated with badger culling compared to the associated beneficial effects, observed while annual culling was ongoing.^{13–16} Available data from the RBCT suggested that only modest reductions in confirmed herd breakdowns would be achievable by badger culling, even if large areas were culled, repeatedly, systematically, and simultaneously (and hence at substantial cost), while small-scale, short-term, or patchy culling was expected to make matters worse; this called into question whether badger culling could meaningfully contribute to the control of cattle TB in Britain.¹ Stakeholders made clear their strongly held and diverse (often directly opposing) views on the form of future TB control policy^{17–19} in a public consultation on badger culling,²⁰ which followed the first publication of results of proactive culling within the RBCT.³ The consultation received more than 47 000 replies,²¹ reflecting the level of public concern in the badger culling issue.

The utility of badger culling as a TB control measure will be influenced not only by the magnitude of its beneficial and detrimental effects, but also by their persistence. The last proactive culls occurred in 2005, but monitoring of cattle TB incidence has continued in and around all trial areas as part

of routine TB surveillance. We therefore compared cattle TB incidence in and around proactively culled areas with that in and around survey-only areas, to determine whether the effects of proactive culling observed during the RBCT persisted following the cessation of culling. We also re-evaluated whether the size of the culled area required to achieve an overall net reduction in the incidence of confirmed herd breakdowns has changed in light of the additional data.

Materials and methods

Trial design

Thirty trial areas, selected on the basis of high cattle TB incidence, were recruited sequentially as 10 matched 'triplets' denoted A–J (see [supplementary material](#) for map). Nearby trial areas were separated by at least 3 km. Each trial area was surveyed for badger activity and then randomly allocated to treatments (except in triplet I, for which security concerns directed a specific allocation) such that each treatment—proactive culling, reactive culling, or no culling ('survey only')—was repeated once within each triplet.

Immediately following treatment allocation, initial proactive culls were conducted on all land for which landholder consent was obtained (see [supplementary material](#) for data on proportion of proactive trial area land for which consent was obtained; note that analyses show that the culling methods used were successful in removing badgers from land for which no access was obtained²). The boundaries of the areas to be culled were delineated (beyond trial area boundaries as necessary) using field survey data to ensure that culling targeted the home ranges of all badgers likely to use farms inside the trial areas; where possible, culling area boundaries followed likely geographical barriers to badger immigration. Badgers were captured in cage traps placed primarily at setts, with no trapping between 1 February and 30 April each year, to avoid killing mothers with dependent cubs below ground.²² Few badgers were found to have trap-related injuries^{23,24} and badger killing (by gunshot) was deemed 'humane' by independent audit.²⁵

Initial culls for each proactive trial area were completed between December 1998 and December 2002, with 'follow-up' culls repeated approximately annually (with longer delays in seven areas incurred due to a nationwide epidemic of foot-and-mouth disease in 2001). The final culls for all 10 proactive areas were completed between May and October 2005. Field surveys indicated that badger activity in trial areas changed according to the culling treatment received,²⁶ with no evidence that treatment comparisons were substantially compromised by illegal culling in survey-only areas.

Once each initial proactive cull was complete, cattle TB incidence inside, and up to 2 km outside, each trial area was monitored using established veterinary surveillance. Effects were sought up to 2 km outside trial areas because culling had been shown to influence badger numbers and movement patterns on this spatial scale.¹¹ The 'during-trial period' was defined as the period from the completion of the initial proactive cull in each triplet, to exactly one year after completion of the last cull in each triplet, when another annual cull would have been conducted had the proactive treatment been continued. The subsequent time period was

termed the 'post-trial period'. Data were available until 6 January 2008, and consisted of accrued totals from 55.7 'triplet-years' in the during-trial period and 14.3 'triplet-years' in the post-trial period (where a 'triplet-year' is one triplet observed for 12 consecutive months). Analyses were restricted to 'confirmed breakdowns' (incidents in which post mortem examination of slaughtered cattle led to detection of TB lesions or culture of *M. bovis*); some breakdowns detected in late 2007 would not have been confirmed in time to be included in these analyses.

Statistical analysis

As in previously published analyses,^{2–5} we used log-linear Poisson regression to compare the numbers of confirmed breakdowns recorded in and around trial areas subjected to the proactive and survey-only treatments. The regression models adjusted for triplet, the log of the number of baseline herds at risk, and the log of the number of confirmed breakdowns recorded in a three-year period before RBCT culling. Where results were stratified by time, a triplet by time interaction term was also included in the model. Confidence intervals (CI) and *p*-values were conservatively adjusted for extra-Poisson overdispersion by using an adjustment factor (the square root of the model deviance divided by the degrees of freedom) in all cases where its value was greater than 1. We used a Chi-square test, summing confirmed herd breakdowns over triplets, to look for any difference between the effect of proactive culling on the incidence of confirmed herd breakdown observed in the last two years of the during-trial period and that observed in the first year post-trial.

Cattle herd locations from the national animal health information system VetNet were used to identify herds inside trial areas. Parallel analyses were performed using the RBCT database to identify herd locations; the results obtained were similar but less consistent (see [supplementary material](#)). Herds up to 2 km outside trial area boundaries were identified more comprehensively using VetNet, because the

RBCT database did not include all farms on neighboring land. Any herds within 2 km of more than one trial area boundary (whether proactive, reactive, or survey-only) were omitted from these analyses. Analyses based on VetNet herd locations include 2659 herds inside trial areas and 2262 herds in neighboring areas, up to 2 km outside trial area boundaries (proactive and survey-only areas combined).

As in previously published analyses,¹ we attempted to estimate the area of land that would need to be targeted by culling to obtain an overall reduction in the incidence of confirmed herd breakdowns (with detrimental effects outside the culled area offset by benefit effects inside). To do this, we investigated whether the effect of culling varied with distance from the trial area boundary, combining the data from the during- and post-trial periods. Any change in effect with distance would have implications for extrapolation to areas larger or smaller than the 100 km² used in the RBCT. Tests for trends in culling effects, with distance from the trial area boundary, were undertaken using weighted least squares on the estimated logarithms of the relative risks associated with each distance category (0–1, 1–2, 2–3, 3–4, and >4 km inside the trial area boundaries or 0–0.5, 0.5–1, 1–1.5, and 1.5–2 km outside the trial area boundaries). These estimates were obtained from models fitted simultaneously to data from distance categories inside the trial area boundaries (with negligible correlation between estimates of effects associated with different distance categories – see [supplementary material](#)), and in a separate model to the data from distance categories outside the trial area boundaries.

Results

Analyses revealed that, during the post-trial period, the incidence of confirmed cattle herd breakdowns was 54% lower inside proactive trial areas than inside survey-only areas (*p* < 0.001 for the null hypothesis of no effect; 95% confidence interval (CI): 39–66% reduction; [Table 1](#), [Figure 2](#)). This result was consistent across all ten triplets

Table 1 Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns inside trial areas

	Proactive effect			Overdispersion	
	Estimate	95% CI	<i>p</i> -Value	Factor	<i>p</i> -Value
1st to 2nd cull	–3.6%	–33.1 to 38.9%	0.85	1.33	0.001
2nd to 3rd cull	–12.9%	–38.8 to 24.2%	0.45		
3rd to 4th cull	–39.6%	–59.3 to –10.3%	0.013		
After 4th cull to end of during-trial period	–31.8%	–48.5 to –9.7%	0.007		
First year of post-trial period	–48.7%	–65.6 to –23.2%	0.001		
Second year of post-trial period	–60.8%	–80.7 to –20.5%	0.009	0.67	0.87
All during-trial period combined	–23.2%	–32.7 to –12.4%	<0.001		
All post-trial period combined	–54.4%	–66.2 to –38.5%	<0.001		
(1st and 2nd year combined)				0.77	0.76
1st cull to 6 January 2008	–30.2%	–38.1 to –21.3%	<0.001	0.73	0.81

95% CI, 95% confidence interval.

Analyses adjust for triplet, baseline number of herds, and historic TB incidence (over three years). Results are split by cull sequence during-trial and by year post-trial and include breakdowns from the initial cull to 6 January 2008. During-trial results include all confirmed breakdowns in the period from completion of the initial proactive cull (in each triplet) to one year after the last proactive cull (in each triplet) and use the January 2007 data download as reported in the ISG Final Report. The post-trial results include all reported confirmed breakdowns from one year after the last proactive cull (in each triplet) to 6 January 2008 and use the download from 6 January 2008. All results are based on locations from the VetNet database.

Table 2 Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns up to 2 km outside the trial area boundaries

	Proactive effect			Overdispersion	
	Estimate	95% CI	p-Value	Factor	p-Value
1st to 2nd cull	43.9%	−8.6 to 126.7%	0.12	1.25	0.007
2nd to 3rd cull	24.4%	−19.0 to 91.0%	0.32		
3rd to 4th cull	20.0%	−25.0 to 92.1%	0.45		
After 4th cull to end of during-trial period	17.3%	−14.4 to 60.9%	0.32		
First year of post-trial period	−16.2%	−45.4 to 28.9%	0.42		
Second year of post-trial period	−30.1%	−67.3 to 49.2%	0.35	1.26	0.13
All during-trial period combined	24.5%	−0.6 to 56.0%	0.057		
All post-trial period combined (1st and 2nd year combined)	−22.7%	−44.3 to 7.3%	0.12	0.91	0.56
1st cull to 6 January 2008	12.5%	−8.5 to 38.3%	0.27	1.33	0.091

95% CI, 95% confidence interval.

Analyses adjust for triplet, number of baseline herds, and historic TB incidence (over three years). Results are split by cull sequence during-trial and by year post-trial and include breakdowns from the initial cull to 6 January 2008. During-trial results include all confirmed breakdowns in the period from completion of the initial proactive cull (in each triplet) to one year after the last proactive cull (in each triplet) and use the January 2007 data download as reported in the ISG Final Report. The post-trial results include all reported confirmed breakdowns from one year after the last proactive cull (in each triplet) to 6 January 2008 and use the download from 6 January 2008. All results are based on locations from the VetNet database.



Figure 1 European badgers (*Meles meles*). In populations undisturbed by culling, badgers are highly social; disruption of this social behaviour is thought to lead to expanded ranging, and increased disease transmission both among badgers and from badgers to cattle. Photos credited to Richard Yarnell.

Table 3 Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns inside trial areas and up to 2 km outside trial areas

	Proactive effect			Overdispersion		<i>p</i> -Value for linear trend
	Estimate	95% CI	<i>p</i> -Value	Factor	<i>p</i> -Value	
Inside treatment areas						
0–1 km inside	–19.5%	–35.4 to 0.3%	0.053	1.33	<0.001	0.083
1–2 km inside	–22.1%	–40.8 to 2.5%	0.074			
2–3 km inside	–36.3%	–55.0 to –9.9%	0.011			
3–4 km inside	–28.9%	–58.0 to 20.2%	0.20			
4–5 km inside	–53.5%	–87.7 to 75.8%	0.26			
Outside trial areas and within 2 km of the trial areas						
0–0.5 km outside ^a	–18.6%	–38.6 to 7.8%	0.15	1.08	0.18	0.46
0.5–1 km outside	28.0%	–3.0 to 68.8%	0.081			
1–1.5 km outside	1.6%	–25.0 to 37.6%	0.92			
1.5–2 outside	16.7%	–16.8 to 63.7%	0.37			

95% CI, 95% confidence interval.

Analyses adjust for triplet, number of baseline herds, and historic TB incidence (over three years). Results are split by distance from the trial area boundary and include breakdowns from the initial cull to 6 January 2008. During-trial results include all confirmed breakdowns in the period from completion of the initial proactive cull (in each triplet) to one year after the last proactive cull (in each triplet) and use the January 2007 data download as reported in the ISG Final Report. The post-trial results include all reported confirmed breakdowns from one year after the last proactive cull (in each triplet) to 6 January 2008 and use the download from 6 January 2008. All results are based on locations from the VetNet database.

^a Treatment areas, within which culling was conducted, were slightly larger than trial areas, and were delineated according to the estimated boundaries of social group territories so that all badgers using farms inside the trial areas could be targeted. As a consequence, some culling was conducted on land immediately outside trial area boundaries (see [supplementary material](#) for parallel analyses).

(the test for overdispersion was not significant, $p = 0.76$). However, this effect in the post-trial period was substantially greater than that observed in the during-trial period (23% reduction (95% CI: 12–33% reduction),² $p = 0.002$ for the null hypothesis of the same effect in the during- and post-trial periods). There was no evidence of a difference between the effect in the latter part of the trial (last two years during-trial) and the first year of the post-trial period ($p = 0.18$). Pooling results over the whole time period (during- and post-trial) showed that incidence was reduced by 30% (95% CI: 21–38% reduction); however, this estimate hides the significant improvement in the impact of proactive culling on the incidence of confirmed cattle herd breakdowns inside proactive trial areas.

On land neighboring proactive trial areas, no detrimental effects were observed in the post-trial period: the incidence of confirmed cattle herd breakdowns was 23% lower (95% CI: 44% lower to 7.3% greater) than that on land neighboring survey-only trial areas ($p = 0.12$ for the null hypothesis of no effect; the test for overdispersion was not significant showing that the effect was consistent across all 10 triplets, $p = 0.56$; [Table 2](#), [Figure 2](#)). However, again, the effect in the post-trial period was significantly different from the detrimental effect (24% greater incidence (95% CI: 1% lower to 56% greater)) observed in the during-trial period ([Figure 2](#), $p = 0.038$ for the null hypothesis of the same effect in the during- and post-trial periods). As with inside trial areas, there was no evidence of a difference between the effect in the latter part of the trial (last two years during-trial) and the first year of the post-trial period ($p = 0.14$ for neighboring areas). Pooling results over the whole time period (during- and post-trial) showed that incidence was increased by 13% (95% CI: 8% reduction to 38% increase); however, this estimate hides the

significant improvement in the impact of proactive culling on the incidence of confirmed cattle herd breakdowns on land neighboring proactive trial areas.

We used these results to predict the effects of culling targeted at areas of different sizes assuming a herd density of 1.25 per km² (roughly that seen in the RBCT areas) and an incidence rate in the absence of culling of eight confirmed breakdowns per 100 herds per annum (a reasonable approximation based on data from the survey-only areas during the RBCT). For example, five annual proactive culls targeting a circular area of 125 km² would be predicted to prevent, on average, only roughly three confirmed breakdowns (14.5 prevented within the 125 km² targeted area and 11.2 induced in the 92 km² neighboring area) in the during-trial period. An additional roughly nine confirmed breakdowns would then be prevented, on average, in the following 12 months (6.8 prevented within the targeted area and 2.1 prevented in the neighboring area). Combining the results from these two time periods gives roughly 12 fewer confirmed breakdowns predicted, on average, in a six-year period including five annual proactive culls with culling then discontinued. For comparison, in the absence of culling 130 confirmed breakdowns would have been expected in the affected area (125 km² targeted plus 92 km² neighboring) over the same six-year time period.

Over the whole time period (during- and post-trial combined), there was a non-significant trend for the benefits of culling to increase as one moves deeper inside trial area boundaries ($p = 0.083$; [Table 3](#)); this was expected as more thorough badger removal was achieved deep inside trial areas.²⁶ There was no evidence of a trend for the effects of culling to change with distance outside the trial area boundary ($p = 0.46$; [Table 3](#)). In extrapolating to areas of

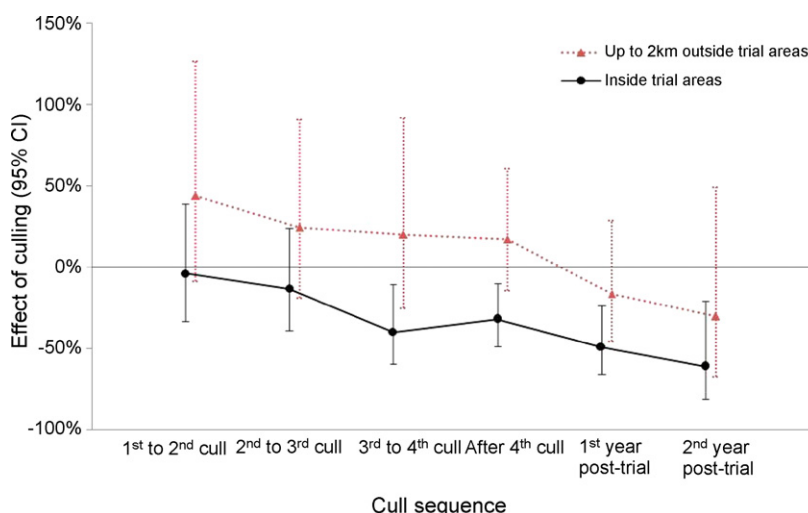


Figure 2 Estimated effects of proactive culling on the incidence of confirmed cattle TB breakdowns inside trial areas and up to 2 km outside trial area boundaries. The estimated effects of proactive culling are stratified by time periods defined by the timings of the culls during the trial, and by year from 1 year after the last proactive cull (post-trial period). The black line shows the effects inside the trial areas and the dotted red line shows the effects in the neighboring areas. These figures are also shown in [Tables 1 and 2](#).

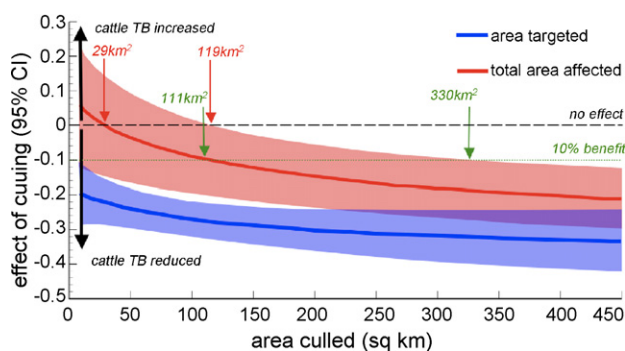


Figure 3 Effects of varying the size of the area targeted for badger culling on the projected impacts on confirmed cattle TB incidence. Red shading shows the 95% confidence interval for the overall impact (combining impacts inside and up to 2 km outside the targeted area) of culling targeted at circular areas of different sizes; blue shading shows the impact inside the targeted area only. The estimated overall effect is for increased incidence when culling targets areas less than 29 km², moving to a decreased incidence when areas of more than 29 km² are targeted. The effect of decreased overall incidence is statistically significant for areas over 119 km². Likewise, on average culling is expected to lead to an overall reduction in cattle TB incidence of $\geq 10\%$ if targeted at areas larger than 111 km², with the expected reduction significantly greater than 10% for areas over 330 km². Calculations assume a trend with distance going deeper inside the trial area from the trial area boundary. To avoid extrapolation beyond the available data, when the beneficial effects of culling are assumed to be linearly dependent on the distance from the boundary, the effect on land more than 4 km inside the trial boundary was assumed to equal to that estimated for such land in the roughly 100 km² RBCT trial areas, even though for much larger culling areas some land will be much further than 4 km from the nearest boundary.

different sizes, we therefore assumed that the beneficial effects of proactive culling inside targeted areas varied with the distance from the boundary, while the detrimental effect on neighboring lands was constant. On this basis, across the combined during- and post-trial periods (including typically five annual proactive culls with culling then discontinued), and considering effects both inside and outside the targeted area, the overall average effect of proactive culling was predicted to lead to a net reduction in the overall incidence of confirmed herd breakdowns, considering the whole affected area, when targeted at circular areas larger than 29 km² ([Figure 3](#)). However, the 95% CI for the average effect across the entire affected area only excluded net increases in the overall incidence of confirmed herd breakdowns, again considering the whole affected area, for culling targeted at circular areas of 110 km² or more (see [supplementary material](#)).

For comparison, we also investigated the size of the area at which culling would need to be targeted to achieve an overall reduction in the incidence of confirmed herd breakdowns (considering effects both inside and outside the targeted area) of 10% or more. On average, such benefits could be expected if culling were targeted at circular areas larger than 111 km² (see [Figure 3](#)). However, the 95% CI around this average value only excluded benefits of $<10\%$ for culling targeted at circular areas of 330 km² or more ([Figure 3](#)). If assumptions were modified so that the treatment effect was constant across the targeted area, the 95% CI excluded benefits of $<10\%$ for circular areas of 443 km² or more (see [supplementary material](#)).

Discussion

Our results show that the reductions in cattle TB incidence achieved through proactive badger culling, as conducted in

the RBCT, persisted for more than one year after culling was discontinued. Beneficial effects inside culling areas increased in magnitude, and detrimental effects were no longer observed on neighboring lands.

The epidemiological mechanisms causing this increase in the beneficial effects of badger culling are uncertain. This is unfortunate, because an insight into these mechanisms would help to predict how long the benefits might be expected to persist.

On the basis of available data, we speculate that the changes observed in cattle TB risks following the cessation of culling reflect changes in the behavior and ecology of badgers, noting that these changes in the risks to cattle were non-significant compared with the final two years of the during-trial period. In the during-trial period, proactive culling was shown to have caused a substantial reduction in badger density inside culled areas, and a smaller reduction on neighboring uncultured lands.^{11,26} In both types of area, badgers ranged more widely;¹¹ the consequently greater opportunities for each infected badger to come into contact with cattle was thought to have caused the detrimental effects on cattle TB outside culling areas, and also to have undermined the beneficial effects inside.³ This expanded ranging behavior also promoted opportunities for transmission among badgers, and probably explains the greater prevalence of *M. bovis* infection observed in badgers on successive culls, which would have further undermined the benefits of reduced badger density.¹²

The subsequent cessation of RBCT culling is likely to have had several consequences for the badger population. It would allow the population to grow, as the abundant, unexploited food source available for badgers would allow high reproductive rates and cub survival.^{27,28} In addition, a stable social organization would be re-established, leading to contraction of home ranges and greatly reduced immigration.²⁹ These two aspects of badger population recovery are likely to occur at different speeds, with changes in badger behavior occurring more rapidly than changes in badger numbers: at two removal sites previously studied in Gloucestershire, badger ranging behavior contracted markedly within two years, but it took 10 years for the original badger densities to be re-established.²⁹ These two effects would have contrasting implications for the incidence of cattle TB: growth of the badger population would be expected to increase the risks of cattle becoming infected from badgers, while the reduction in badger mobility would reduce those risks. We suspect that the reductions in confirmed herd breakdowns recorded in and around proactive areas following the suspension of culling, noting that these estimates were non-significantly different from those observed in the final two years of the during-trial period, reflect contractions in badger ranging at a time when badger numbers were still suppressed by past culling. If this explanation is correct, it suggests that the benefits observed in the first years post-culling will dissipate as badger numbers increase. Continued monitoring will allow testing of this prediction; the timescale on which benefits would be expected to disappear cannot yet be determined. It is likewise not possible to predict how culling over different periods of time, or at different intervals, would have influenced the results, since the outcome would depend upon a complex array of factors including badger movements, dispersal, reproduction, and trappability, all of which are likely to have

changed in response to repeated culling as well as showing strong seasonal variation.

When considering the available data in their entirety, our analyses suggest that the overall reduction in the incidence of confirmed herd breakdowns associated with widespread badger culling remains modest (e.g., on average only 12 confirmed breakdowns prevented over six years by five annual culls targeting a 125 km² area, compared with 130 confirmed breakdowns expected in the absence of culling). As published previously, culling that is small-scale, patchy, or short-term is likely to increase, rather than reduce, TB risks to cattle.^{1–3,5} In the during-trial period,¹ the 95% CI indicated that culling could be expected to lead to a net reduction in the overall incidence of confirmed herd breakdowns if targeted at circular areas greater than 265 km²; the addition of data from the post-trial period reduced this minimum area to 119 km². While this area is considerably smaller than previously estimated, achievement of an effective cull across such an area would still require a coordinated and sustained effort; furthermore the economic costs of implementation (when considering the impact over the whole affected area) would exceed the savings achieved through reduced breakdown incidence (based on the estimated costs of performing culling and experiencing a breakdown^{1,6}).

Our results suggest that the cessation of annual proactive badger culling influenced the dynamics of *M. bovis* infection in cattle and badgers. However, it must be stressed that these results refer only to the time course of badger culling that was implemented in the RBCT, within areas selected for inclusion in the RBCT based on their historic high TB incidence rates in cattle herds: no reliable predictions can be made concerning what would have happened had annual culling been continued for longer, or halted sooner. The reductions in confirmed herd breakdowns we describe from the post-trial period must be considered in the context of the smaller reduction (and indeed increased number of breakdowns in neighboring areas) observed when culling was ongoing. Continued monitoring will determine how long the beneficial effects last, and will thus provide a measure of the overall capacity of badger culling (as conducted in the RBCT) to reduce cattle TB incidence.

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Conflict of interest statement: HEJ and CAD have a research contract with the Department of Environment, Food and Rural Affairs (Defra) to analyze post-trial TB incidence data from cattle herds within RBCT areas. CAD and RW were formerly members of the Independent Scientific Group on Cattle TB (ISG) for which they received fees for time spent on ISG work and reimbursement of expenses from Defra. HEJ was formerly employed as a research assistant working with the ISG at Imperial College London.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2008.04.001.

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