Eurosurveillance

Volume 13, Issue 42 - 16 October 2008

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Rapid communications

Unknown disease in South Africa identified as arenavirus infection

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Introduction

On 12 September 2008, a tourist guide organising safari trips, residing in Lusaka, Zambia, was evacuated in a critical condition to Johannesburg, South Africa. She was admitted to a clinic where she died on 14 September about 10 days after the onset of symptoms. The symptoms included a prodromal phase with fever, myalgia, vomiting, diarrhoea, followed by rash, liver dysfunction and convulsions [1]. Cerebral oedema was detected on scan examination. No laboratory specimen was available for investigation.

The paramedic who had cared for the index case during her evacuation to Johannesburg developed prodromal symptoms similar to the index case. He was hospitalised on 27 September. His condition deteriorated and he died on 2 October. An intensive care unit nurse who cared for the index case in Johannesburg developed similar flu-like symptoms and was hospitalised on 1 October. Her condition deteriorated on 4 October and she died on 5 October of acute respiratory distress syndrome. In both cases, the incubation period is estimated to have been about one week. On 13 October, the World Health Organization (WHO) posted a website update informing about a fourth case affecting a nurse who had been in contact with the paramedic [2].

On 12 October 2008, the National Institute for Communicable Diseases (NICD) in South Africa provided preliminary evidence that the causative agent of the disease was a virus from the Arenaviridae family [3]. Specimens were shipped to the United States Centers for Disease Control and Prevention (CDC) in Atlanta for additional investigations.

Arenavirus taxonomy

Arenaviruses are enveloped viruses (about 120 nm diameter) with a bi-segmented negative strand RNA genome. The typical image in electronic microscopy showing grainy ribosomal particles ("arena" in latin) inside the virions gave the name to this family of viruses. The prototype is the Lymphocytic Choriomeningitis (LCM) virus, isolated in 1933 in North America from a human case with aseptic meningitis. Cases caused by LCM occur worldwide. Other arenaviruses causing hemorrhagic fevers were reported in South America, causing sporadic cases or limited outbreaks: Junín virus in 1958 in Argentina, Machupo virus in 1963 in Bolivia, Guanarito virus in 1990-1991 in Venezuela, Sabia virus in 1990 in Brazil and more recently Chapare virus in 2004 in Bolivia [4]. In West Africa, Lassa virus was identified in Nigeria in 1969. It causes thousands of cases each year in Sierra Leone, Liberia, Guinea and Nigeria. However, only limited data are available to assess the real incidence of Lassa fever in Africa.

Clinical symptoms of arenavirus infections, treatment and vaccine

Two types of clinical presentations are described: neurological and haemorrhagic fever. However, asymptomatic arenavirus infection may be frequent. The incubation period is about 10 days (3-21 days). LCM viruses cause aseptic meningitis or meningoencephalitis with an overall case fatality <1%. Foetal infections can result in congenital abnormalities or death. Transmission of arenaviruses via organ transplantation has been documented; immunosuppressed recipient patients can develop fatal haemorrhagic fever-like disease [5,6]. The Lassa viral haemorrhagic fever usually presents as a non-specific illness with symptoms including fever, headache, dizziness, asthenia, sore throat, pharyngitis, cough, retrosternal and abdominal pain, and vomiting. In severe forms, facial oedema is associated with haemorrhagic conjunctivitis, moderate bleeding (from nose, gums, vagina, etc.) and exanthema. Neurological signs may develop and progress to confusion, convulsion, coma and death. Severe prognosis is associated with a high viraemia, elevated aspartate aminotransferase (AST) liver enzymes, bleeding, encephalitis and oedema. There is a very high risk of foetal mortality in pregnant women during the third trimester of pregnancy. Case fatality rates range from 5 to 20% for hospitalised cases. Clinical symptoms of infection by arenaviruses in South America are similar to those described for Lassa fever in Africa.

Ribavirin has been shown to be an effective treatment for Lassa fever, especially when started within the first six days of illness [7,8]. There is currently no vaccine for Lassa fever but several candidates are under development studies with successful trials in primates [9]. One available vaccine is licensed in Argentina for Junín virus.

Reservoir of arenavirus and transmission

Arenaviruses are associated with rodents, their natural hosts. Some of these viruses can be transmitted to humans by contact with faeces, urine, blood or saliva of infected rodents or with dust containing infective particles. In South America, Machupo and Junín viruses were identified in Calomys rodent, and Guanarito virus was found in a Sigmodon cotton rat [10]. In West Africa, Mastomys natalensis (a peridomestic rodent) is the reservoir of Lassa virus. Its geographic distribution is much wider in sub-Saharan Africa that the presently known area of Lassa transmission [11]. Other

arenaviruses such as Mopeia virus in Mozambique had been isolated from rodents without evidence of disease in humans [10].

Fatal nosocomial and laboratory infections by arenaviruses have been reported. Contamination occurs via direct contact with body fluids or via droplets. Since the 1970s special procedures for handling these viruses (now categorised as class 4 agents) have been put in place, including the building of dedicated biosafety laboratories (BSL-4), with containment equipment for all activities involving the virus, infectious or potentially infectious body fluids or tissues.

Conclusion

In the cluster reported here, four cases have been identified including an index case and three cases of subsequent nosocomial transmission among health workers. The clinical presentation was consistent with neurological symptoms of arenavirus infection. As the incubation period for arenaviruses is up to three weeks, secondary cases may still be identified as part of the follow-up of contacts established in response to this event.

The professional activities of the index case could have favoured possible exposure to rodent excreta in a rural area.

This is the first identification of an arenavirus causing human disease in a southern African country. Further laboratory investigation will allow characterisation of the virus associated with this outbreak and its relation with the existing Lassa virus present in West Africa.

Since 1969, at least 24 cases of Lassa fever are known to have been exported outside Africa, including 16 cases imported to Europe [12,13]. However, in none of these cases has secondary transmission resulted in a symptomatic disease.

Acknowledgements

We gratefully acknowledge the openness, the collaboration and the information provided by the National Institute for Communicable Diseases in South Africa, as well as the International and Tropical Department of the French Institute for Public Health Surveillance (Institut de veille sanitaire, InVS).

References

1. National Institute for Communicable Diseases (NICD). Outbreak of an acute disease, ex-Zambia. Communicable Diseases Communique volume 7 alert 1; October 2008. Available from: http://www.nicd.ac.za/pubs/communique/2008/NICDSpecialCommOct_alert01.pdf
2. WHO website: http://www.who.int/csr/don/2008_10_13/en/index.html
3. ProMED-mail. Undiagnosed fatalities - S. Africa ex Zambia (7): arenavirus. Archive Number 20081012.3234. 12 October 2008. Available from: http://www.promedmail.org/pls/otn/f?p=2400:1001:3702875069304837::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000,74346
4. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, et al. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. PLoS Pathog. 2008;4(4):e1000047.
5. Brief report: Lymphocytic choriomeningitis virus transmitted through solid organ transplantation - Massachusetts, 2008. MMWR Morb Mortal Wkly Rep. 2008 Jul 25;57(29):799-801
6. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med. 2008;358(10):991-998.
7. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, et al. Lassa fever. Effective therapy with ribavirin. N Engl J Med. 1986;314(1):20-6.
8. World Health Organization. Lassa fever - fact sheet. April 2005. Available from: http://www.who.int/mediacentre/factsheets/fs179/en/
9. Lukashevich IS, Carrion R Jr, Salvato MS, Mansfield K, Brasky K, Zapata J, Cairo C, Goicochea M, Hoosien GE, Ticer A, Bryant J, Davis H, Hammamieh R, Mayda M, Jett M, Patterson J. Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates. Vaccine. 2008 Sep 26;26(41):5246-54.
10. Charrel RN, de Lamballerie X. Arenaviruses other than Lassa virus. Antiviral Res. 2003 Jan;57(1-2):89-100.
11. Fichet-Calvet E, Lecompte E, Koivogui L, Soropogui B, Doré A, Kourouma F, et al. Fluctuation of abundance and Lassa virus prevalence in Mastomys natalensis in Guinea, West Africa. Vector Borne Zoonotic Dis. 2007;7(2):119-128
12. Macher AM, Wolfe MS. Historical Lassa fever reports and 30-year clinical update. Emerg Infect Dis. 2006 May;12(5):835-7.
13. Unit for Surveillance and Communication, Unit for Preparedness and Response, Editorial team. E-alert 24 July: Case of Lassa fever imported into Germany from Sierra Leone. Euro Surveill. 2006;11(30):pii=3008. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3008

This article was published on 16 October 2008.

Citation style for this article: Zeller H, Leitmeyer K, Varela Santos C, Coulombier D. Unknown disease in South Africa identified as arenavirus infection. Euro Surveill. 2008;13(42):pii=19008. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19008

Rapid communications

Report on the measles situation in Portugal

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A measles case, imported from the United Kingdom (UK), was reported in Portugal in late August 2008. Previously, measles cases imported from Romania, and import-related cases, were reported in 2005. There was no transmission to the autochthonous Portuguese population.

Background

Measles vaccination in Portugal started with a national campaign in 1973 and was included in the National Vaccination Program (PNV) in 1974. Since 1990, it consists of two doses of measles vaccine in the form of the combined measles, mumps and rubella vaccine (MMR), with the first dose at the age of 15 months and the second dose at the age of 11-13 years, The latter was changed to the age of 5-6 years in 2000 [1,2].

In 2001/2002, the seropositivity of the general Portuguese population against measles was of 95.2%, with a vaccination coverage of 95-98% and a seroconversion efficacy of 90-95% [1]. The high vaccination coverage has since been maintained and the last two confirmed cases of reported indigenous measles in the autochthonous Portuguese population occurred in 2002 and 2003, respectively.

In August 2008, and previously in 2005, imported and import-related measles cases affecting children of foreign origin, have been reported in Portugal. Viral transmission was contained in both instances and the virus was not transmitted within the autochthonous Portuguese population.

Case in 2008

In late August 2008, the Portuguese Directorate-General of Health was notified of a possible measles case in the Algarve Region, located in the south of the country. The notification was first made to the local health authority by a private paediatrician who had seen a six-year-old British child on 25 August, following onset of measles-like symptoms on 21 August and development of a rash on 23 October. Epidemiological investigations were immediately initiated and included laboratory detection and characterisation of the virus.

The child had travelled from the UK on 17 August, on vacation with both parents and two siblings aged 11 and 14 years. Both parents were vaccinated against measles. The three children were unvaccinated, and the patient's siblings received a post-exposure dose of MMR on 26 August.

The case was confirmed by detection of anti-measles IgM in the serum and by the presence of viral genome in a urine specimen. The virus was classified as genotype D4, and 449 out of 450 nucleotides encoding the C-terminal region of the nucleoprotein were identical to the genotype D4 strain MVs/Enfield.GRB/14.07 presently circulating in the UK [3].

This finding, and the fact that during the viral incubation period the child was still in England, identifies the case as imported from the UK.

Cases in 2005

In June 2005, an imported measles case had been reported, resulting in an outbreak affecting six children in two Romanian communities living in Portugal. Epidemiological investigations identified as the probable index case a child who had travelled from Romania to Portugal a week before rash onset. The other five children were epidemiologically linked to the index case and all presented clinical symptoms compatible with measles virus infection. Their measles immunisation status was unknown. All six cases were laboratory-confirmed by detection of anti-measles IgM in the sera and the presence of viral genome in oral and urine specimens. The virus was classified as genotype D4. A 200 nucleotide region encoding the C-terminal portion of the nucleoprotein was sequenced and showed 100% similarity to a genotype D4 viral strain concurrently circulating in Romania.

Discussion

The elimination of indigenous measles has been achieved in Portugal by steady high vaccination coverage (ca. 95%) and by strict implementation of disease control measures. The high anti-measles seropositivity of the general Portuguese population and the rapid investigation and control of the described imported and import-related cases, have permitted to contain viral transmission and contributed to the absence of reported cases within the autochthonous Portuguese population since 2003.

Measles did not become endemic again in Portugal in 2005, or in 2008, and the phylogenetic analysis of both imported viruses revealed different geographical origins.

The importation of measles cases remains a public health concern, because pockets of low vaccination coverage (85-94%) still persist in the population, thereby maintaining a possibility of acquiring the disease [1]. Globally, importation of measles cases and subsequent outbreaks as reported by several European countries in the last years, and with a very high incidence in 2008

[4-11], can compromise the goal of measles elimination in Europe by 2010 set by the World Health Organization Regional Office for Europe [12].

Acknowledgements

On behalf of the professionals who diagnosed, treated, notified, investigated and controlled the situations described: Private paediatrician in Albufeira; Paediatrics Departments of the Faro and Guimarães hospitals; Local Public Health Services of Albufeira, Guimarães and Vila Nova de Famalicão; Public Health Department of the Regional Health Administration of the Algarve; Public Health Department of the Regional Health Administration of the North; Disease Prevention and Control Division of the Directorate-General.

References

1. Andrade HR, Gíria M. Measles virus [Vírus do Sarampo]. In: Avaliação do Programa Nacional de Vacinação. 2º Inquérito Serológico Nacional Portugal Continental 2001-2002, Direcção Geral da Saúde. Lisboa: DGS; 2004. p. 191-205. [In Portuguese].
2. Portuguese Directorate-General for Health. Supplementary measles vaccination. Circular No: 10/DSCS/DCPD. 5 June 2008. Available from: http://www.euvac.net/graphics/euvac/pdf/plan_portugal_eng.pdf
3. Health Protection Agency. Confirmed measles cases in England and Wales – an update to end-May 2008. Health Protection Report. 2008;2(25). [Accessed August 16, 2008]. Available from: http://www.hpa.org.uk/hpr/archives/2008/news2508.htm#meas0508
4. Muscat M, Christiansen AH, Böttiger BE, Plesner A, Glismann S. A cluster of measles cases in Denmark following importation, January and February 2008. Euro Surveill. 2008;13(9):pii=8050. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8050
5. Nieto-Vera J, Masa-Calles J, Dávila J, Molina-Font J, Jiménez M, Gallardo-García V, et al. An outbreak of measles in Algeciras, Spain, 2008 – a preliminary report. Euro Surveill. 2008;13(20):pii=18872. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18872
6. Follin P, Dotevall L, Jertborn M, Khalid Y, Liljeqvist JÅ, Muntz S, et al. Effective control measures limited measles outbreak after extensive nosocomial exposures in January-February 2008 in Gothenburg, Sweden. Euro Surveill. 2008;13(30):pii=18937. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18937
7. Filia A, De Crescenzo M, Seyler T, Bella A, Ciofi Degli Atti ML, Nicoletti L, et al. Measles resurges in Italy: preliminary data from September 2007 to May 2008. Euro Surveill. 2008;13(29):pii=18928. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18928
8. Thierry S, Alsibai S, Parent du Châtelet I, on behalf of the investigation team. An outbreak of measles in Reims, eastern France, January-March 2008 - a preliminary report. Euro Surveill. 2008;13(13):pii=8078. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8078
9. Pfaff G, Mezger B, Santibanez S, Hoffmann U, Maassen S, Wagner U, et al. Measles in south-west Germany imported from Switzerland - a preliminary outbreak description. Euro Surveill. 2008;13(8):pii=8044. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8044
10. Heathcock R, Watts C. Measles outbreaks in London, United Kingdom - a preliminary report. Euro Surveill. 2008;13(15):pii=18829. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18829
11. Eurosurveillance editorial team. Measles once again endemic in the United Kingdom. Euro Surveill. 2008;13(27):pii=18919. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18919
12. Muscat M, Bang H, Glismann S. Measles is still a cause for concern in Europe. Euro Surveill. 2008;13(16):pii=18837. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18837

This article was published on 16 October 2008.

Citation style for this article: Gíria M, Rebelo-de-Andrade H, Fernandes T, Pedro S, Freitas G. Report on the measles situation in Portugal. Euro Surveill. 2008;13(42):pii=19010. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19010

Rapid communications

Measures taken to reduce the risk of West Nile virus transmission by transplantation in Italy

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For the first time in Italy, two patients with meningoencephalitis were diagnosed with West Nile virus (WNV) infection in September 2008 [1]. The patients live in the Bologna and Ferrara provinces of Emilia Romagna where WNV infections had previously been noted in horses, crows and magpies [2].

The Italian National Transplant Centre (CNT), which is responsible for the procurement, processing and distribution of organs and tissues in Italy, has now reviewed the risks of transmission of WNV by organ, tissue and cell transplantation and, taking into account the advice and recommendations of the relevant authorities in other countries, issued guidance to the transplant community.

Background

Although there are no known cases of WNV transmission by tissue transplantation, both blood transfusion and organ transplantation have resulted in transmissions [3-6]. Transmission by transplantation of tissues such as bone, heart valves, skin and corneas is theoretically possible, particularly when the tissues have been minimally processed and/or they contain blood.

European Union Directive 2006/17/EC, Annex 1, defines the criteria to be applied for the selection of tissue and cell donors and Annex 2 defines the testing requirements [7]. Annex 1 states that donors must be excluded from donation if there is evidence of "risk factors for transmissible diseases on the basis of a risk assessment, taking into consideration donor travel and exposure history and local infectious disease prevalence". This requirement is reflected in the CNT's Guidelines for the Procurement, Processing and Distribution of Tissues for Transplantation (19/06/2007), which demand the exclusion of donors who have "a risk of infection associated with travel to endemic areas or exposure to infective agents that cannot be excluded by testing".

The Food and Drug Administration (FDA) in the United States issued draft, non-binding guidance for blood, cell and tissue donations in April 2008 [8]. They recommend that blood specimens from all human cell and tissue donors be tested year-round for WNV by individual donor Nucleic Acid Testing (NAT) using a licensed screening NAT test, and that only donors whose specimens are non-reactive may be considered eligible.

The American Association of Tissue Banks has raised concerns about the necessity for universal testing seeing as there are as yet no reports of transmission by tissue transplantation. They have also raised concerns regarding the reliability of the kits that have been licensed for use with post-mortem samples: They report an unacceptable rate of abnormal initial tests that give a weak positive result but are negative when repeated for the same sample [9].

Apart from these concerns, it is relevant that in the case of the first donor that transmitted WNV by organ transplantation, two serum samples collected at the time of admission did not contain any detectable WNV IgM antibody or nucleic acid. Neither did a serum sample obtained from the patient on the following day, after receipt of transfusions contain detectable levels of WNV nucleic acid. However, serum and plasma samples collected a day later at the time of organ recovery yielded WNV nucleic acid in a quantitative PCR, and WNV could be recovered in culture [4]. In the second case of transmission by organ transplantation, the donor tested negative for WNV RNA, although serum samples were positive for WNV IgG and IgM [5].

These findings underline the problems that can arise when relying on testing and the importance of accurate documentation of the donor's history in the prevention of donor-transmitted infections such as West Nile fever.

It is notable that in the United Kingdom, 18,700 blood donors returning from high-incidence WNV areas during the epidemic season were tested between mid-June 2004 and the end of November 2005 and no positive result was obtained [10]. It can be concluded that the risk of transmission by those who have travelled to affected areas is very low.

Guidance issued for transplantation in Italy

In the light of the recently reported infections, and taking a precautionary approach, the Italian National Transplant Centre has issued guidance that all potential donors of organs, tissues and cells from the Bologna and Ferrara provinces in the Emilia-Romagna region should be tested to exclude infection. Where there is evidence of infection, organs, tissues and cells will not be used.

In the rest of Italy and in the other Emilia-Romagna provinces, he following rules apply:

- Investigation of the history of potential **tissue donors** will include enquiries regarding a possible overnight stay in the provinces of Bologna and/or Ferrara during the previous 28 days. If a potential donor has visited one of these provinces, they will

not be considered eligible for donation, unless laboratory test results for WNV are negative;
- For **organ donors**, a case by case evaluation is conducted in order to assess the infection risk, which is acknowledged to be very low, taking into account the nature and benefits of transplantation and the health status of the patient on the waiting list.

Discussion

In the case of organ donation, decisions have to take into account the shortage of organs available for transplant and the great, usually life-saving, benefit that can result from this type of transplantation. The available time is very limited and it may not always be possible for WNV testing to be performed in a particular area of Italy where a donor is identified in time before the transplantation would need to proceed. Under such circumstances, the risk posed by a potential donor who may have spent a night in a WNV-affected area in the previous month and has had no symptoms of infection would be very low and would probably not justify depriving the recipients of the opportunity for transplantation. For this reason, it is necessary to consider each case individually, weighing the risks and potential benefits that face each individual recipient in a balanced and pragmatic way.

In the case of tissue donation, the potential donor pool is much larger and shortages are therefore not a major challenge in the system, particularly in Italy where tissue donation rates are high. In general, tissue transplants result in improved quality of life and are rarely life-saving, so it is important to maintain risk at a very low level. On the other hand, many donated tissues are processed by washing, freezing, freeze-drying and in some cases subjected to gamma irradiation or other types of sterilisation. The tissues that are not highly processed, such as corneas, heart valves and skin, contain very little blood. The risk of an infected tissue transmitting a virus is therefore significantly lower than for blood or organs. Overall, it is considered appropriate to take a precautionary approach to the selection of tissue donors until there is a clearer picture of the extent of the problem.

The guidance described here will be reviewed should further infections be reported, also taking into account changing seasons.

References

1. Rossini G, Cavrini F, Pierro A, Macini P, Finarelli AC, Po C, et al. First human case of West Nile virus neuroinvasive infection in Italy, September 2008 – case report. Euro Surveill. 2008;13(41):pii=19002. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19002
2. Macini P, Squintani G, Finarelli AC, Angelini P, Martini E, Tamba M, et al. Detection of West Nile virus infection in horses, Italy, September 2008. Euro Surveill. 2008;13(39):pii=18990. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18990
3. Centers for Disease Control and Prevention (CDC). Update: West Nile Virus Screening of Blood Donations and Transfusion-Associated Transmission --- United States, 2003. MMWR Morb Mortal Wkly Rep. 2004;53(13):281-4.
4. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348(22):2196-203.
5. Centers for Disease Control and Prevention (CDC). West Nile Virus Transmissions in Organ Transplant Recipients – New York and Pennsylvania, August – September 2005. MMWR Morb Mortal Wkly Rep. 2005;54(40):1021-3.
6. Centers for Disease Control and Prevention (CDC). West Nile Virus Transmission through Blood Transfusion – South Dakota, 2006. MMWR Morb Mortal Wkly Rep. 2007 Feb 2;56(4):76-9.
7. European Commission. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells2006/17/EC. Brussels; 2006. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF
8. Center for Biologics Evaluation and Research. Draft Guidance for Industry: Use of Nucleic Acid Tests to Reduce the Risk of transmission of West Nile Virus from Donors of Whole Blood and Blood Components Intended for Transfusion and Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). US Department of Health and Human Services, Food and Drug Administration; April 2008 Available from: http://www.fda.gov/cber/gdlns/natwnvhctp.htm#iv
9. American Association of Tissue Banks. Comments in response to the FDA publication " Draft Guidance for Industry: Use of Nucleic Acid Tests to Reduce the Risk of Transmission of West Nile Virus from Donors of Whole Blood and Blood Components Intended for Transfusion and Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)". McLean, Virginia; July 2008. Available from: http://www.aatb.org/files/fdacomments72508.pdf
10. The Standing Advisory Committee on Transfusion Transmitted Infections. Position Statement West Nile Virus 05 February 2008. Joint UKBTS/NIBSC Professional Advisory Committee. Available from: http://www.transfusionguidelines.org.uk/index.asp?Publication=DL&Section=12&pageid=801

This article was published on 16 October 2008.

Citation style for this article: Nanni Costa A, Grossi P, Porta E, Venettoni S, Fehily D. Measures taken to reduce the risk of West Nile virus transmission by transplantation in Italy. Euro Surveill. 2008;13(42):pii=19009. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19009

Surveillance and outbreak reports

Tuberculosis in a shopping centre, Portugal, 2004-5

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Genotyping enables to confirm or exclude a tuberculosis (TB) cluster. Excluding the link between cases is particularly important in countries with intermediate/high incidence of TB where the emergence of several TB cases in a particular location in space or time (higher than the expected) could be explained by chance alone.
During 2004 and 2005, five TB cases occurred in five shops of a Portuguese shopping centre which employed a total of about 1000 workers. After an epidemiological survey, 52 close contacts were identified and screened. Latent tuberculosis infection was diagnosed in 10 contacts (eight family members and two work colleagues of cases). Genotyping of the *Mycobacterium tuberculosis* isolates revealed no link between the cases. For this reason no screening of all staff of the shopping centre was carried out. However, close contacts (52) and all fellow workers (1000) were kept under surveillance for two years, and no additional cases were diagnosed.
The present analysis demonstrates that the exclusion of a chain of ongoing transmission by genotyping for the investigation of a cluster is cost-effective from the perspective of the public health service, because it allows to avoid unnecessary large scale screening operation and instead to direct resources to more effective measures of TB control.

Introduction

Tuberculosis (TB) remains a serious problem worldwide. In Portugal, the incidence of TB is 29.4 per 100,000 inhabitants per year [1], higher than the European Union average of 17 per 100,000 [2]. Contact screening is mostly aimed at identifying family, social and work contacts of cases [3,4]. It is often difficult to decide how far to proceed with screening, particularly if several cases coincide in time and space, which in intermediate/high incidence countries can be due to chance only. A good understanding of the factors affecting the transmission of the disease in the community may result in avoiding the diagnosis of false clusters and directing the resources to more effective measures of TB control.

Molecular typing can help clinicians and public health practitioners to identify or exclude clusters of recently acquired tuberculosis [5,6]. *Mycobacterium tuberculosis* isolates from space or time clusters are expected to show identical or very closely related genotypic patterns [7]. IS6110 restriction fragment length polymorphism (RFLP) has been used as the "gold standard" method for more than a decade [7]. This method provides the highest discriminatory power among *M. tuberculosis* typing techniques, showing sufficient variability to distinguish unrelated strains.

This paper is based on a retrospective review of the investigation of a suspected time–space cluster of cases of TB. During 2004 and 2005, five cases of active tuberculosis were identified among employees of a shopping centre with 172 shops and a total of approximately 1000 workers in Vila Nova de Gaia, south of Porto in north-west of Portugal. The TB diagnosis was based on culture and identification of *M. tuberculosis*. The isolates were confirmed to be fully sensitive. The patients were voluntarily tested for human immunodeficiency virus (HIV) infection and all were negative. All five patients were started on directly observed therapy short-course (DOTS) and all have completed the treatment.

A number of common features were found in the five patients. They all lived in neighbouring districts near the shopping centre and frequented the same food and leisure places. This raised the question of what size of population should be subject to screening.

Material and methods

An epidemiological survey was performed in order to identify the daily activities of all TB patients. Home, transport, workplace and social settings of the TB cases were described (size of place, ventilation, time and length of exposure, etc.) and contacts were identified. This information was put together to disclose all possible links between the five cases, taking into account the presumed infectious period of the cases, known contacts between the cases, residence, transport used, spatial distribution of the shopping centre and social activities.

Close contacts were defined as household members, co-workers (of the same shop), and persons who had spent more than a cumulative contact time of eight hours in a confined environment with the case during the symptomatic phase (before the diagnosis and the beginning of treatment). All close contacts of TB patients were offered the screening programme, including symptom questionnaire, tuberculin skin testing (TST) and chest X-ray (CXR).

Distant contacts were defined as employees of other shops of the same shopping centre with no known contact with the TB patients or other persons who had only had sporadic contact lasting less than 8 hours with the cases during the symptomatic phase.

All identified contacts (both close and distant) were followed for a two-year period and special attention was given to the identification

of all new cases in the area covering the districts of residence of the cases and the shopping centre to discard possible links with these five cases. The follow-up included clinical examination of all the close contacts screened (52 close contacts) and surveillance of all employees of the shopping centre, including those not considered to be close contacts and therefore not screened. The period of follow-up was two years, as it is known that 10% of cases of latent TB infection develop active tuberculosis, and 5% do so two years after infection [7].

In order to identify the link between the cases and thus provide evidence for further public health decisions, genotyping techniques were used to analyse the clinical strains. Molecular strain typing was performed using the standard method IS6110 RFLP [7]. We used a combination of external and internal standards as positive controls, including a reference strain of *M. tuberculosis* named Mt14323. The latter gives 10 approximately evenly spaced bands of known size. This combination of markers allows extremely precise band molecular size determinations and permits computerised comparisons between strains.

The costs of the screening programme were calculated based on the values published in the Portuguese official journal of legal acts – Diário da República (Republic Diary) 113 1ª série/B published 12 June 2006. All costs are reported in euros and presented in Table 1. TST licensed for Portugal is PPD RT 23, 2 T.U. from the Danish Statens Serum Institute. The delivered price for 10 glass vials, each containing 1.5 ml RT 23, is 149.99 euros. From the 1.5 ml vials, we withdraw 10 test doses of 2 T.U. The charge for the RFLP analysis made by the reference laboratory (Instituto Nacional de Saúde, Laboratório de Tuberculose, Porto) is 149.64 euros.

Table 1

Base-case estimates used in cost analysis of the investigation of tuberculosis cases in a shopping centre in Vila Nova de Gaia, Portugal, 2004-5

Public health service (PHS) procedures and other costs (in euros)	Base-case cost estimate (in euros)	Source
Tuberculin skin test	15	PHS*
Chest radiography	9.80	PHS*
Medical consultation (doctor, 25 minutes)	30	PHS*
Restriction fragment length polymorphism (RFLP)	149.64	Laboratory provider

PHS* Costs of public health service procedures are per test/procedure as listed in Diário da República (Republic Diary) 113 1ª série/B published 12 June 2006

Results

The five cases' mean age was 31 years (range 28 to 36 years). They all had pulmonary active tuberculosis, based on microbiologic identification. The median time between the onset of symptoms and diagnosis was four months (range 2-9 months). In practice, there was no delay between diagnosis and treatment initiation (diagnoses took one day).

The cases lived in neighbouring boroughs around the shopping centre but did not have any social contact outside the workplace, not even in public transport. They all worked in different shops within the shopping centre but two pairs of cases worked on the same floor. Three cases frequented the same restaurant regularly and four cases went to the same leisure place once a week, but they never met on those occasions.

Fifty-two close contacts (mean 10 per case, range 7-16 contacts) were identified and examined. Close contacts were identified among family (20), close friends (15) and work colleagues (17). Latent tuberculosis infection was diagnosed in 10 contacts (eight among family members and two among work colleagues) and treatment was provided (OR=2.2, 95% CI: 0.4-22, p=0.466). No additional cases of TB infection were diagnosed.

All cultures obtained from cases were regrown on Lowenstein Jensen culture medium slants but only four out of five produced colonies, resulting in the loss of one strain. When growth was considered to have attained an optimal biomass, cells were harvested and inactivated. IS6110 RFLP results revealed that at least four of the five TB cases were caused by strains with different hybridisation patterns thus discarding the possibility of transmission of the disease inside the shopping centre. The fingerprints of the four *M. tuberculosis* isolates investigated are shown in the Figure. Taking into consideration the definition of cluster as two or more strains sharing the same IS6110 RFLP pattern, none of the strains included in this study were clustered, because all patterns were different as demonstrated in Figure.

Therefore, we concluded that the TB cases were not linked and decided not to extend the screening programme beyond the close contacts.

The cost of TB screening in our public health service is 54.8 euros per patient. The total cost of screening of the 17 close contacts identified among the shopping centre employees was 931.6 euros. The additional cost of genotyping of the four *M. tuberculosis* isolates was 598.56 euros. Had we screened all workers of the shopping centre (about 1000 people) we would have spent 54,800 euros.

No additional cases of TB were diagnosed during the two years of follow-up, either among the close contacts who had been screened or other employees of the shopping centre.

Table 2

Characteristics of cases of tuberculosis identified among employees of a shopping centre in Vila Nova de Gaia, Portugal, 2004-5 (n=5)

Case	Date of diagnosis	Age at time of diagnosis(years)	Sex	TB site	AFB sputum smears	PCR result	Symptoms
1	May 2004	29	Female	Pulmonary	Positive	Positive	Yes
2	July 2004	33	Female	Pulmonary	Positive	Positive	Yes
3	Sep 2005	28	Female	Pulmonary	Positive	Positive	Yes
4	Oct 2005	36	Female	Pulmonary	Positive	Positive	Yes
5	Dec 2005	31	Male	Pulmonary	Positive	Positive	Yes

Discussion

A disease cluster is a local anomaly in the data where the observed incidence for a particular location in space and/or particular time interval appears to be different (higher) from the expected, based on the assumption of a uniform disease distribution among persons at risk, irrespective of time or location [9].

When the possibility of a TB cluster arises, particularly in a public space, screening procedures must be extended to a larger population. However, when making decisions about the extent of contact tracing and screening we need to weigh the chance of missing potentially exposed individuals against causing unnecessary anxiety in a large number of people involved.

Of the 17 work colleagues screened, two (12%) had latent TB infection (LTBI). Among family and close friends, LTBI was detected in eight out of 35 contacts (23%). In the general Portuguese population, LTBI prevalence has been estimated at 15% [1], which is higher than the rate observed in the co-workers but lower than that found among family and close friends. Thus the number of LTBI cases detected among work colleagues was not higher than expected in the general population. Family contacts, on the other hand, were found to be at increased risk for LTBI.

Extending the screening procedures beyond close contacts raises questions regarding the efficacy and the real benefit especially in a population with intermediate/high incidence of tuberculosis. Universal screening is no longer advised. We must therefore direct our efforts at identifying the individuals at risk and understanding the local mechanisms of tuberculosis transmission in order to define the best strategy to control the disease.

The investigation described in this paper benefited from evolving technologic solutions in the field of genotyping. IS6110 RFLP revealed that none of the four isolates obtained shared the same genotype, which ruled out the hypothesis of an outbreak.

With the exclusion of a link between the cases and a prevalence of LTBI lower than expected among the other workers of the same shop, we decided not to extend the screening procedures to the rest of the staff. Thus we managed to save about 1000 screenings.

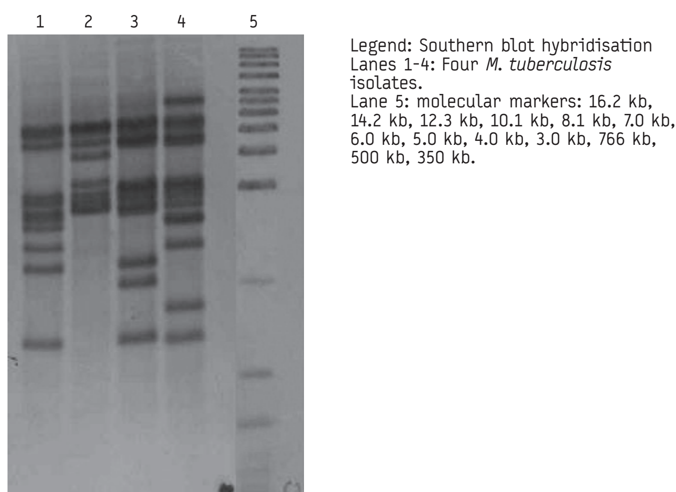
The follow-up provided evidence that our decision was right because no other TB case was diagnosed among the employees of the shopping centre or residents of the neighbouring area.

A thorough assessment based on clinical and laboratory diagnosis combined with genotyping of all *M. tuberculosis* isolates is recommended for the confirmation or exclusion of an outbreak. Usually, the literature describes the usefulness of genotyping techniques in confirming a cluster, but it is also very important when it can exclude a link between the cases. In countries with intermediate/high prevalence of TB, resources must be directed towards the optimisation of active TB treatment and the screening of contacts at risk.

The present analysis demonstrates that the combination of genotyping with the traditional TB screening procedures in the investigation of a cluster, from the perspective of the public health service, allows to save financial and human resources. In the situation presented, it allowed to exclude a link between the different cases and concentrate the resources on the individuals who were really at risk. We would therefore suggest that in countries with intermediate/high TB incidence genotyping should be performed whenever there is a suspicion of a cluster to confirm or exclude a chain of ongoing transmission and thus decide on the size of population to be screened.

Figure

IS6110 restriction fragment length polymorphism (RFLP) result of *M. tuberculosis* isolates obtained from four cases of tuberculosis in a shopping centre in Vila Nova de Gaia, Portugal, 2004-5



Legend: Southern blot hybridisation
Lanes 1-4: Four *M. tuberculosis* isolates.
Lane 5: molecular markers: 16.2 kb, 14.2 kb, 12.3 kb, 10.1 kb, 8.1 kb, 7.0 kb, 6.0 kb, 5.0 kb, 4.0 kb, 3.0 kb, 766 kb, 500 kb, 350 kb.

References

1. Programa Nacional de Luta Contra a Tuberculose, Direcção Geral da Saúde. Tuberculose: Ponto da Situação em Portugal em 2006, dados preliminares em Março de 2007. [in Portuguese]. Available from: http://www.dgs.pt/upload/membro.id/ficheiros/i009162.pdf
2. EuroTB and the national coordinators for tuberculosis surveillance in the WHO European Region. Surveillance of tuberculosis in Europe. Report on tuberculosis cases notified in 2006. Saint-Maurice, France: Institut de veille sanitaire; March 2008. Available from: http://www.eurotb.org/rapports/2006/full_report.pdf
3. Duarte R, Amado J, Lucas H, Sapage JM, Portuguese Society of Pulmonology. [Treatment of latent tuberculosis infection: update of guidelines, 2006] [Article in Portuguese] Rev Port Pneumol. 2007;13(3):397-418.
4. Veen J. Microepidemics of tuberculosis: the stone-in-the-pond principle. Tuber Lung Dis. 1992;73(2): 73-6.
5. Huang HY, Jou R, Chiang CY, Liu WC, Chiu HJ, Lee JJ. Nosocomial transmission of tuberculosis in two hospitals for mentally handicapped patients. J Formos Med Assoc. 2007;106(12):999-1006.
6. Saleiro S, Santos AR, Vidal O, Carvalho T, Torres Costa J, Agostinho Marques J. [Tuberculosis in hospital department health care workers] [Article in Portuguese]. Rev Port Pneumol. 2007 Nov-Dec;13(6):789-99.
7. National TB Controllers Association / CDC Advisory Group on Tuberculosis Genotyping. Guide to the Application of Genotyping to Tuberculosis Prevention and Control. Atlanta, GA: US Department of Health and Human Services, CDC; June 2004. Available from: http://www.cdc.gov/tb/genotyping/manual.htm
8. Blower SM, McLean AR, Porco TC, Small PM, Hopewell PC, Sanchez MA, et al. The intrinsic transmission dynamics of tuberculosis epidemics. Nat Med. 1995;1(8):815-821.
9. Aamodt G, Samuelsen SO, Skrondal A. A simulation study of three methods for detecting disease clusters. Int J Health Geogr. 2006;5:15.

This article was published on 16 October 2008.

Citation style for this article: Duarte R, Miranda A, Braga R, Carvalho A, Rola J, Marques A, Barros H. Tuberculosis in a shopping centre, Portugal, 2004-5 . Euro Surveill. 2008;13(42):pii=19004. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19004

Surveillance and outbreak reports

Serologic and virologic surveillance of avian influenza in Nigeria, 2006-7

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Since January 2006, H5N1 avian influenza has affected Nigeria's poultry population causing enormous loss of resources. The current circulating virus is a potential candidate for pandemic influenza which may severely affect the human and animal population worldwide especially in the resource-poor countries. In this study, we report on our field and laboratory surveillance efforts in Nigeria. A total of 1,821 tissue samples, 8,638 tracheal swabs, 7,976 cloacal swabs and 7,328 avian sera were analysed over a period of two years, with 312 positive results.* We recovered 299 isolates of highly pathogenic avian influenza virus H5N1 mainly from the diagnostic samples of poultry kept in backyard, small scale and free range farms. This finding emphasised the role played by these farming systems in the dissemination of avian influenza in Nigeria and highlights the need for a continued surveillance in humans since human-animal interaction is a key feature in Africa. Furthermore, there is a need for the strengthening of border controls. Since October 2007, there has been no reported and confirmed outbreak of avian influenza in Nigeria.

Introduction

In late 1996, a farm in Guangdong, China was affected by infections with highly pathogenic avian influenza (HPAI) virus H5N1 [1,2]. Since the time of these reports, several countries in Asia (n=17), Europe (n=27), the Middle East (n=7) and Africa (n=11) have reported infection or re-infection of poultry flocks and/or wild and migratory birds [3].

In parts of the continents that reported infection, with the exception of Europe, it has been documented that the virus is becoming entrenched in the poultry populations and many clades and sub-clades are emerging [4]. Several hundred human infections (n=372) including 235 fatalities have similarly been confirmed [5], and most of these human infections have been linked to exposure to domestic poultry [6].

The expanding geography (infection of new locations), biology (acquisition of new biological properties) and ecology (adaptation to new host range) of H5 influenza viruses necessitated that every country should actively search for H5 avian influenza viruses within its territories. Nigeria, a country with an estimated human population of over 140 million, first reported infection in poultry in January 2006 [7], and in humans in January 2007 [5], and since that time, efforts to carry out active surveillance for the influenza viruses have been intensified by the national authority. Poultry production is a key economic activity in Nigeria. It contributes significantly to the family income, especially in peri-urban and poor rural communities [8]. The effect of growing urbanisation the rural, peri-urban and urban poultry production and on human-animal interaction has previously been reported [9]. Backyard poultry production thrives in view of the level of poverty and the economic return associated with the venture. Free-range systems of poultry production are also widespread in various parts of the country [10].

Due to H5N1 avian influenza infection in Nigeria, millions of poultry have been destroyed and one human death has occurred. A recent serological survey in humans in those administrative regions in Nigeria that were most heavily affected by HPAI H5N1 showed that, despite the widespread infection in the poultry population, human infection is rare [11]. In this report, we describe our surveillance efforts in Nigeria and discuss the role of poultry and backyard flocks and their implications for humans vis-à-vis our laboratory findings.

Materials and methods

Poultry surveillance on farms and live bird markets

System 1 (October to December 2007). Based on available records, a stratified sampling with cluster sampling within each strata was adopted that included locations around previously infected farm premises and live bird markets as well as locations with suspected outbreaks and dense poultry populations. Each state of Nigeria was visited three times at intervals of two weeks, and samples were taken at two new locations during every visit. At each location, cloacal, tracheal and serum samples were taken from 29 birds, and six moribund, clinically ill or dead birds were purchased. All samples were transported in appropriate media and the cold chain was maintained throughout the activities.

System 2 (May to July 2008). The national active surveillance covered all 36 Nigerian states and the Federal Capital Territory (FCT), irrespective of whether or not HPAI H5N1 infections had been reported from the area, but was carried out in two parts: Part A of the targeted live bird market surveillance covered only the states with infections (25 states and FCT), while part B covered the 11 states without infections. This targeted surveillance programme is still ongoing.

System 3 (February 2006 to December 2007). While these activities were going on, additional routine diagnostic samples

(mostly tissue samples) were submitted to the National Veterinary Research Institute (NVRI) or collected in the field by the NVRI staff.

National surveillance programmes and team

In response to the outbreak of H5N1 influenza in the poultry population in 2006, the Nigerian government set up an inter-ministerial committee comprising health (Federal Ministry of Health), veterinary/agricultural (Federal Ministry of Agriculture and Rural Development) and information personnel (Federal Ministry of Information) to tackle the growing problem. Several routine surveillance efforts were jointly carried out at various times by the national teams in collaboration with representatives from the Food and Agricultural Organisation of the United Nations (FAO), the United States Centers for Disease Control and Prevention (US CDC), the World Organisation for Animal Health (OIE), the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE) and others. Teams were regularly dispatched to suspected farms nationwide to collect samples and identify infected birds, advise on compensations and carry out cullings.

Sample collection, virus isolation and serology from avian species

Following sample collection, post mortem examinations were conducted on birds acquired moribund, dead or freshly killed, and on tracheas, lungs, livers, spleens, brains, hearts, intestines as well as intestinal contents were collected in sterile containers.

Virus isolation was done in 9-11-day-old embryonated chicken eggs according to standard protocols [12]. The eggs were candled daily to determine viability and dead eggs were removed and kept at +4oC. All eggs were opened aseptically and the allantoic fluids (ALF) were spot-tested by haemagglutination test. The chorio-allantoic membranes (CAM) of positive eggs were tested by agar-gel immunodiffusion (AGID) to detect influenza A virus group antigen.

Haemagglutination-inhibition (HI) test was conducted to determine the virus subtype. All negative ALF were further passaged in a second set of embryonated chicken eggs. Any samples negative after the second passage were declared negative. As of May 2008, no isolates of influenza A virus have been obtained from the second passage**.

Serological assays including AGID test using the H5 antigen and HI test using standardized H5, H7 and H9 panels of antigens (OIE reference laboratory for Newcastle disease virus and avian influenza, Padova) were conducted on all sera submitted to the laboratory.

Molecular analysis

Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) were carried out. A cascade-type analysis was performed starting with the gene for the viral matrix protein (M). Every positive result was subjected to an RT-PCR for the haemagglutinin gene (HA) of subtype H5 and an additional RT-PCR for the N1 gene was done for all HA-positive cases. The following oligonucleotide primers were used: M forward: 5'-AGA TGA GTC TTC TAA CCG AGG TCG-3'; M reverse: 5'-TGC AAA AAC ATC TTC AAG TCT CTG-3'; H5 forward: 5'-CCT CCA GAR TAT GCM TAY AAA ATT GTC-3'; H5 reverse: 5'-TAC CAA CCG TCT ACC ATK CCY-3'.

Conventional RT-PCR has been shown to detect titre as low as 3 EID50 (Fifty percent egg infectious dose). In addition, our results were confirmed by the OIE reference laboratory for avian influenza and Newcastle disease, Padova, Italy.

Human sero-epidemiological surveillance

Several locations (poultry farms, live bird markets) with suspected or confirmed HPAI H5N1 infections were visited (between 21 March and 3 April 2007) following the compilation of a list of affected areas by the Federal Ministry of Agriculture and Rural Development in Nigeria. Specifically, a total of 295 poultry workers (76% farm workers, 15% market workers, 5% poultry cullers and 4% veterinarians), from 83 farms and four live bird markets in Kano state, and 25 laboratory workers were included in the surveillance.

In addition, surveillance in humans had been carried out by Ortiz et al. between 21 March and 3 April 2006 [11]. In that study, human sera had been collected with the informed consent of participating individuals. In addition, serum samples had been collected from people potentially exposed to the HPAI H5N1 virus, including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples had been transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera had been prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera had been tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. For details see Ortiz *et al.* [11]*.

Results

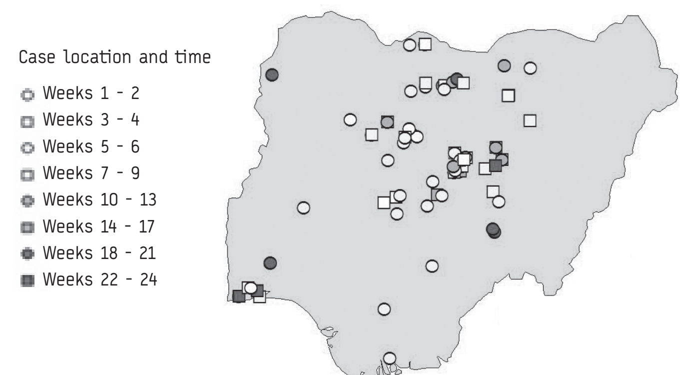
Poultry sero-surveillance

In the period between 2006 and 2007, farms located in 25 Nigerian states and the FCT reported poultry infections with HPAI H5N1 virus. The geographical distribution of the positive cases is shown in Figure 1.

Details of the results are shown in the tables below. During the two-year study period from January 2006 to December 2007, a total of 1,205 suspected routine diagnostic samples, 8,638 cloacal swabs, 7,976 tracheal swabs, 7,328 sera and 616 carcasses were received either from the field staff or directly from the farmers.

Figure 1

Temporal and geographical distribution of highly pathogenic avian influenza H5N1 poultry cases, Nigeria, January-June 2006 (n=113)



The surveillance in birds was carried out in the whole country with particular attention paid to the infected locations and live bird markets around them. Further 186 cases occurred between June and December 2007, bringing it to a total of 299 cases, but the overall geographical distribution did not change, with additional infections happening only in already affected locations.

The samples submitted as part of routine surveillance (system 3) yielded 300 positive results (Table 1).

Table 2 shows the results of the national surveillance using stratified a sampling procedure covering farms and live bird markets in the 36 Nigerian states and FCT (system 1).To date, all of these 10,961 samples have been negative.

For the targeted live bird market surveillance (system 2), results are available for part A covering only the 25 infected states and the FCT (Table 3). A total of 13,597 samples were analysed, of which 12 were found to be positive. The targeted live bird market surveillance for the 11 states without report of avian influenza infections (part B) is ongoing.

In the period from January 2006 to December 2007, 299 isolates of HPAI H5N1 were obtained and characterised. The haemagglutinin genes of 52 isolates have been sequenced and deposited in the GenBank and EMBL databases [13]. All of the positive isolates that were characterised belonged to clade 2.2. Efforts to genetically characterise more of the remaining isolates are currently underway.

Table 1

Avian diagnostic samples tested in Nigeria between 2006 and 2007

	Suspected total number	Positive samples
Diagnostic samples (tissues/swabs) tested in 2006	619	145
Diagnostic samples (tissues/swabs) tested in 2007	586	154 + 1*
Total	1,205	299 + 1*

Note: 52 isolates have been fully sequenced and are published [13]. A large majority (98%) of the isolates originated from farms.
1* represents a sample from Benin republic diagnosed in Nigeria.

Table 2

National active surveillance covering the 36 states and the Federal Capital Territory, Nigeria, October 2007 to July 2008 (n=10,961 samples)

Samples Collected	Number analysed	Number positive
Tracheal swabs	4,253	0
Cloacal swabs	3,608	0
Sera	3,100	0

Table 3

Targeted live bird market surveillance covering 25 states and the Federal Capital Territory, Nigeria, October-November 2007 (n=13,597 samples)

Samples Collected	Number analysed	Number positive
Tracheal swabs	4,385	3
Cloacal swabs	4,368	0
Sera	4,228	6
Carcasses and moribund birds	616	3

Tables 1-3 reveal a certain pattern in that H5N1 influenza virus isolates were obtained mainly from routinely submitted diagnostic samples and live bird markets. Following infection of farms, farmers promptly report outbreaks to the NVRI or other appropriate government agencies since this will ensure payment of compensation. However, we are aware that the level of education may affect reporting in certain circumstances and our systems may have inadvertently missed some outbreak situations. It is also very likely that viruses that escape detection at the farm level will get to the live bird market and can be detected there. These two locations (farms and live bird markets) are important in the epidemiology of avian influenza viruses in Africa.

Figure 2 gives an overview on HPAI H5N1 outbreaks in the years 2006 and 2007 as determined by the routine diagnostic poultry surveillance.

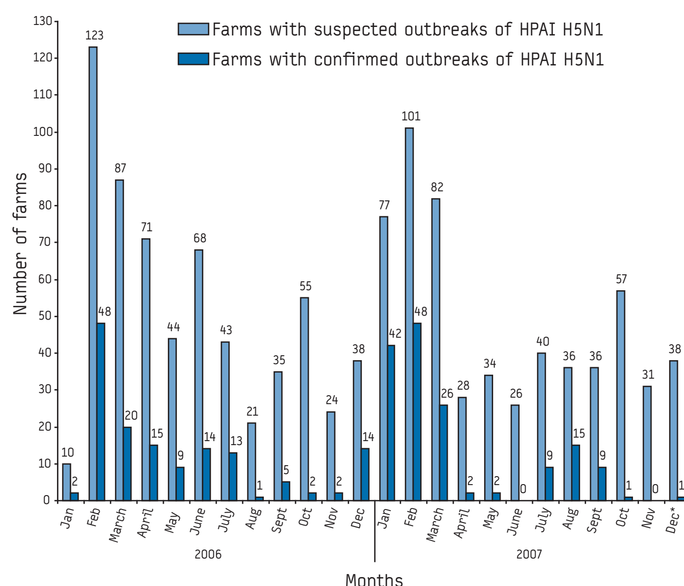
The overall rate of confirmed outbreaks was 24.8%. The peaks of infection around January and February in both years may be linked to poultry movement which is usually on the increase around festive periods (December/January). The peaks in the June/July 2006 and July/August 2007 period similarly represent the times when seasonal guineafowl eggs are available. The same period is accompanied by sale of commercial poultry due to a surplus in the egg market caused by the cheaper guineafowl eggs.

Human sero-surveillance

As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test despite the degree of possible exposure to H5N1 influenza virus [11]*.

Figure 2

Suspected and confirmed outbreaks of HPAI H5N1 from routine diagnostic samples of avian species in Nigeria, 2006-2007 (n=1,205 reports and 299 confirmations)



2007 Dec*: The only isolate in that month originated from Benin Republic.
HPAI: Highly pathogenic avian influenza.

Discussion

Before the first occurrence of avian influenza in Nigeria, active surveillance on wild fowl and migrating birds was conducted between September and November 2005 (results not shown) at the Nguru-Hadejia wetlands covering an area of about 4,125 km2. Similar surveillance was done in the same period in the high risk agroecological/farming areas and live poultry markets, but failed to detect H5 or H7 avian influenza virus.

However, after the first avian influenza outbreak in Nigeria in January 2006, surveillance efforts in the period between January, 2006 and December, 2007 yielded a total of 299 Nigerian isolates of HPAI H5N1. Mutations at antigenic sites were identified in the haemagglutinin genes of the viruses, the significance of which need to be confirmed by further analyses. The implications of these mutations for human and animal health is yet unknown [13]. Although the H5N1 virus has not yet adapted to effectively infect humans, there remains a potential pandemic threat in view of continuous infections on farms in the West African sub-region. Furthermore, there is a need to carry out routine surveillance for other influenza viruses in human and animals, since a recent report using animal models indicated that the H9N2 influenza virus showed increasing pandemic potential [14].

We are aware that our surveillance systems are subject to certain limitations. Firstly, the systems were limited and not all locations within each state were considered. In addition, some bias may be caused by the fact that the surveillance of birds may not be possible in difficult terrains. However, we made every effort to give priority to locations that serve as points of aggregation of poultry products from many locations.

We may have underdetected some cases in view of the availability of more robust and sensitive analytic systems like real time RT-PCR, and are currently making an effort to put in place such an analytic system. It was also difficult to get paired serum samples in most locations since farmers were free to dispose of their birds without regards to the on-going surveillance.

Despite these limitations, we think that this nationwide effort is critical and important since Sub Saharan Africa faces many challenges of controlling and eradicating H5N1 in poultry and implementing a good surveillance system for H5N1 in humans.

The human sero-epidemiological survey reported by JR Ortiz *et al.* did not detect any human H5N1 infections in Nigeria [11]*. This result is similar to the data recorded in previous studies in Cambodia (0/351) and Guangdong, China (1/110) [15,16]. This probably confirms that the virus has not yet adapted to effectively infect humans.

Although the human serosurveillance was negative, human H5N1 infections in Nigeria cannot be excluded. It is common practice in the northern part of the country, for reasons of culture, religion and poverty, to bury a deceased person within 24 hours of death, sometimes without ascertaining the cause of death through post mortem and detailed laboratory examinations. The only human case in Nigeria, which was officially reported by the World Health Organization on 3 February 2007, was diagnosed following a thorough investigation of a fever complicated by respiratory distress which finally led to death. It is important to ensure in the future that at least diagnostic specimens are collected before burial for proper retrospective analysis. Since it is beyond the mandate of NVRI to do a nation-wide serosurveillance in humans, the Nigerian Federal Ministry of Health, human medical practitioners, virologists and immunologists are encouraged to carry out a similar study in humans in Nigeria and parts of the West African sub-region.

Globalisation can affect animal and human health and change the disease ecology especially in those countries that presently claim to be free from HPAI infection in humans and animals [17], and risk assessment studies have shown that the European Union and parts of North America are at high risk of infection with animal diseases, in particular those originating from Africa [18-21]. These countries will need to strengthen their borders with respect to animal disease controls.

To date, the majority of the HPAI H5N1 cases in Europe has been introduced through wild birds. The source of contamination as well as the movement pattern of these wild and migratory birds needs to be studied more critically in order to exclude cross-continent infection of a potentially pandemic influenza virus.

Since October 2007, there has been no confirmed outbreak in Nigeria despite the on-going intensive surveillance. This situation has helped to stabilise the Nigerian poultry industry and has had a positive psychological effect on consumers. However, the continued absence of HPAI H5N1 will depend on sustained surveillance of poultry farms and live-bird markets, changed agricultural practices and a heightened biosecurity system entrenched in the farming system in Nigeria. Cross-continent collaborative research is encouraged and a network of funding systems, especially from the rich countries, to support research and diagnosis in developing economies like Nigeria will be greatly valued.

** Note added in proof: Since the time of submission of this report, the FAO laboratory has recently (June and July, 2008) isolated and molecularly characterised new HPAI virus isolates obtained from live bird markets and from outbreaks in farms in a total of four Nigerian states. While the viruses from two states, Kano and Katsina, (isolated from farms) belonged to the old clade (2.2) circulating in Nigeria, the isolates from two other states, Gombe and Kebbi, belonged to a new sublineage of clade 2.2, EMA3, that is novel to the African continent. This sublineage was previously circulating in Europe (Italy), Asia (Afghanistan) and the Middle East (Iran) in 2006.

* Erratum: The following amendments were made to correct the fact that supporting data on sero-surveillance in humans had mistakenly not clearly been labelled as cited from a previous publication: The sentence “Limited human sero-surveillance involving 320 individuals was also carried out but yielded no positive results” was removed from the abstract. The paragraph “Surveillance in humans was carried out between 21 March and 3 April 2006 [11]. Human sera were collected with the informed consent of participating individuals. In addition, serum samples were collected from people potentially exposed to the HPAI H5N1 virus including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples were transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera were prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera were tested by microneutralisation assay and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. Details of the tests have been reported comprehensively in another paper [11].” was changed to “In addition, surveillance in humans had been carried out by Ortiz et al. between 21 March and 3 April 2006 [11]. In that study, human sera had been collected with the informed consent of participating individuals. In addition, serum samples had been collected from people potentially exposed to the HPAI H5N1 virus, including laboratory workers, veterinarians and culling staff that agreed to participate in the sero-survey. The blood samples had been transported on ice to the laboratory (Institute of Human Virology, Abuja). Sera had been prepared in the Human Virology Laboratory, Abuja, and split in two aliquots, one of which was kept for the Federal Ministry of Health while the other one was sent to the US CDC for H5N1 serologic testing. The human sera had been tested by microneutralisation assay

and a modified horse red blood cell haemagglutination-inhibition (HRBC H-I) assay. For details see Ortiz et al. [11]." The sentence "None of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test ..." was changed to "As previously reported, none of the 320 human serum samples tested was positive for H5N1 avian influenza by micro-neutralisation assay or HRBC H-I test ... [11]." The sentence "The human sero-epidemiological survey reported in this study did not detect any human H5N1 infections in Nigeria." was changed to "The human sero-epidemiological survey reported by JR Ortiz et al. did not detect any human H5N1 infections in Nigeria [11]."

References

1. Tang X, Tian G, Zhao J, Zhou KY. Isolation and characterization of prevalent strains of avian influenza viruses in China [In Chinese]. Chin J Anim Poult Infect Dis. 1998;20:1–5.
2. Shortridge KF, Zhou NN, Guan Y, Gao P, Ito T, Kawaoka Y, et al. Characterization of Avian H5N1 Influenza Viruses from Poultry in Hong Kong. Virology. 1998;252(2):331-42.
3. World Organisation for Animal Health. Update on Highly Pathogenic Avian Influenza in Animals (Type H5 and H7). Available from: http://www.oie.int/downld/avian%20influenza/A_AI-Asia.htm. Accessed on 18 January 2008.
4. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus, Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z, Hayden FG, Nguyen DH, et al. Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med 2008;358(3):261-73.
5. World Health Organization. Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO. Available from: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2008_03_11/en/index.html. Accessed on 11 March 2008.
6. De Martin S, Nicoll A, Coulombier D. A/H5N1 in the European Union: current levels of risk to humans, and responding to human cases and outbreaks. Euro Surveill. 2006;11(11):pii=656. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=656
7. Joannis T, Lombin LH, De Benedictis P, Cattoli G, Capua I. Confirmation of H5N1 avian influenza in Africa. Vet Rec. 2006;158(9):309-10.
8. Central Bank of Nigeria. Annual Report and Statement of Account. Abuja: Central Bank of Nigeria; 2004.
9. Fasina FO, Bisschop SP, Webster RG. Avian influenza H5N1 in Africa: an epidemiological twist. Lancet Infect Dis. 2007;7(11):696-7.
10. Adene DF, Oguntade AE. The structure and importance of the commercial and village based poultry industry in Nigeria. Rome: Food and Agricultural Organization; 2006. Available from: http://www.fao.org/docs/eims/upload//214281/poultrysector_nga_en.pdf
11. Ortiz JR, Katz MA, Mahmoud MN, Ahmed S, Bawa SI, Farnon EC, et al. Lack of Evidence of Avian-to-Human Transmission of Avian Influenza A (H5N1) Virus among Poultry Workers, Kano, Nigeria, 2006. J Infect Dis. 2007; 196(11):1685-91.
12. World Organisation for Animal Health. Avian influenza. In: Manual of diagnostic tests and vaccines for terrestrial animals. Web edition. Available from: http://www.oie.int/eng/normes/mmanual/A_00037.htm. Accessed on 15 July 2006.
13. Fasina FO, Bisschop SP, Joannis TM, Lombin LH, Abolnik C. Molecular characterization and epidemiology of the highly pathogenic avian influenza H5N1 in Nigeria. Epidemiol Infect. 2008;doi:10.1017/S0950268808000988.
14. Wan H, Sorrell EM, Song H, Hossain MJ, Ramirez-Nieto G, Monne I, et al. Replication and transmission of H9N2 influenza viruses in ferrets: Evaluation of pandemic potential. PLoS ONE. 2008;3(8):e2923.
15. Vong S, Coghlan B, Mardy S, Holl D, Seng H, Ly S, et al. Low frequency of poultry-to-human H5N1 virus transmission, southern Cambodia, 2005. Emerg Infect Dis. 2006;12(10):1542-7.
16. Wang M, Di B, Zhou D-H, Zheng B-J, Jing H, Lin Y-P, et al. Food market with live bird as source of avian influenza. Emerging Infect Dis. 2006;12(11):1773-5.
17. Marano N, Arguin PM, Pappaioanou M. Impact of globalization and animal trade on infectious disease ecology. Emerg Infect Dis. 2007;13(12):1807-9.
18. Wooldridge M, Hartnett E, Cox A, Seaman M. Quantitative risk assessment case study: smuggled meats as disease vectors. Rev Sci Tech. 2006;25(1):105-17.
19. Van Borm S, Thomas I, Hanquet G, Lambrecht B, Boschmans M, Dupont G, et al. Highly pathogenic H5N1 influenza virus in smuggled Thai eagles, Belgium. Emerg Infect Dis. 2005;11(5):702-5.
20. Mcquiston JH, Carl M, Mitruka K, Bateman J, Palumbo G, Corsino C, et al. Illegal Bushmeat Importation into the United States, 2005–2006. Abstract at the International Meeting on Emerging Diseases and Surveillance. Vienna, Austria. 23-25 February 2007.
21. Wallace RG, Fitch WM. Influenza A H5N1 Immigration Is Filtered Out at Some International Borders. PLoS ONE. 2008;3(2):e1697.

This article was published on 16 October 2008.

Citation style for this article: Joannis TM, Meseko CA, Oladokun AT, Ularamu HG, Egbuji AN, Solomon P, Nyam DC, Gado DA, Luka P, Ogedengbe ME, Yakubu MB, Tyem AD, Akinyede O, Shittu AI, Sulaiman LK, Owolodun OA, Olawuyi AK, Obishakin ET, Fasina FO. Serologic and virologic surveillance of avian influenza in Nigeria, 2006-7. Euro Surveill. 2008;13(42):pii=19007. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19007

News

Use of hand disinfection reduces absenteeism from day care centres

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A study carried out in day care centres in ten counties in southern and central Sweden showed that using hand disinfection with ethyl alcohol reduced absenteeism among children due to illness by 12%. The study, recently published in *Acta Paediatrica* [1], was planned and coordinated by the strategy group for rational use of antibiotics and reduced antibiotic resistance (Strama), the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet, SMI), and the public health department in Örebro County Council, Sweden. It estimated that the cost of caring for sick children could be decreased by about 200 million SEK per year. The cost to introduce hand disinfection gel in day care centres across Sweden was estimated at 10 million SEK per year.

Initially, the study included nearly 3,000 children at 60 day care centres during a 30-week period from 2004 to 2005. A complete evaluation was available for 1,431 children in 29 day care centres. Neighbouring day care centres were paired up, with one of the centres using hand disinfection after hand washing (intervention group), while the other centre using soap and water only (control group). Absenteeism due to illness was measured by means of the centres' attendance lists and compared between the intervention and control groups. In the intervention group, staff and children used hand disinfection gel containing 70% ethyl alcohol following each hand wash with soap, on average between two and six times per day, after bathroom use, before eating, and when dirty.

There are other published studies connecting hygiene routines with absenteeism at day care centres in the United States, Finland, Canada, and Australia. In those studies, hand disinfection was only one of several components. The Swedish study is the first study to evaluate hand disinfection exclusively, a simple measure that is inexpensive to introduce.

After the study had ended, several day care centres chose to continue the use of the disinfection gel.

References

1. Lennell A, Kühlmann-Berenzon S, Geli P, Hedin K, Petersson C, Cars O, et al. Alcohol-based hand-disinfection reduced children's absence from Swedish day care centers. Acta Paediatrica. Epub 1 Oct 2008; DOI: 10.1111/j.1651-2227.2008.01057.x.

This article was published on 16 October 2008.

Citation style for this article: Lennell A, Fredlund H. Use of hand disinfection reduces absenteeism from day care centres. Euro Surveill. 2008;13(42):pii=19006. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19006

News

European Commission invites experts to join scientific committees

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control, Stockholm, Sweden

On 5 September 2008, the European Commission adopted Decision 2008/721/EC for establishment of a revised Scientific Risk Assessment Advisory Structure. The Commission is now inviting applications from scientists to join this advisory structure of scientific committees and database of experts. For the official text for this call for expression of interest please visit:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2008:245:0023:0025:EN:PDF

Depending on their expertise and availability, scientists may apply for membership in one or more of five bodies: Scientific Committee on Consumer Safety; Scientific Committee on Health and Environmental Risks, Scientific Committee on Emerging and Newly Identified Health Risks; Pool of Scientific Advisors; and Database of Experts.

This call for expression of interest is open to scientists worldwide. The Commission seeks to appoint members from different geographic regions and to achieve a gender balance.

Applicants for membership in a Scientific Committee or the Pool of Scientific Advisors are expected to have at least 10 years of professional experience. Members are appointed to a Scientific Committee for a period of three years and may not serve more than three consecutive terms. Scientific advisors are appointed to the Pool for a period of five years and their term may be renewed. The closing date for submitting applications is 31 October 2008.

Membership in the Database of Experts is open to scientists wishing to support the activities of the Scientific Committees on an ad hoc basis. There is no closing date for application and candidates are not required to have a specific number of years of professional experience.

For information on eligibility criteria, workload, indemnities, application procedure and to download an application form, please visit: http://ec.europa.eu/health/ph_risk/committees/call_expression_en.htm

This article was published on 16 October 2008.

Citation style for this article: Eurosurveillance editorial team. European Commission invites experts to join scientific committees . Euro Surveill. 2008;13(42):pii=19005. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19005