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Mycobacterium fortuitum from lesions of slaughtered pigs in Ibadan, Nigeria

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Summary

To ascertain the cause of tuberculous-like lesions in pigs slaughtered in a local abattoir in Ibadan (south-western Nigeria), a total of 516 pigs were inspected over a period of four months, 18 of which had gross lesions suggestive of tuberculosis at post-mortem. Mycobacterial culture and molecular typing (GenoType® Mycobacterium CM [Common Mycobacteria] assay) analysis were used to identify and confirm the mycobacteria species responsible for these lesions.

Results show that 2.3% (12/516) of the animals screened were infected with mycobacteria; *Mycobacterium fortuitum* was confirmed in 33.3% (4/12) of these cases.

As far as the authors are aware, this is the first report confirming the isolation of *M. fortuitum* in slaughtered pigs in Nigeria. There is a need to improve on necessary preventive and control measures that will reduce potential sources of mycobacterial infections in pig-rearing herds. These infections may also have public health implications, especially to workers in the pig industry.

Keywords

GenoType® Mycobacterium CM assay – Ibadan – Meat inspection – Molecular technique – Mycobacteria – *Mycobacterium fortuitum* – Nigeria – Pigs – Porcine – Public health.

Introduction

Tuberculosis in pigs causes large economic losses to farmers all over the world (22). The common causes of tuberculosis in pigs are members of the *Mycobacterium tuberculosis* and *M. avium* complexes. However, other species of mycobacteria, known as non-tuberculous mycobacteria (NTM), may participate in the formation of tuberculous-like lesions in domestic and wild pigs (18, 22). These species, which are also known as atypical or opportunistic mycobacteria or conditionally pathogenic mycobacteria, are, as follows:

– *M. fortuitum*
– *M. gordonae*
– *M. terrae*
– *M. chelonae*
– *M. smegmatis*
– *M. phlei*
– *M. scrofulaceum.*

One of the most commonly notified NTM sources for domestic pigs is drinking water for animals (18). Water may not only become a transport medium, but also serve as the main reservoir for the majority of NTM species. Contaminated bedding, particularly straw, sawdust, wood shavings, shredded paper and other materials made of wood, may be significant NTM sources for pigs (15, 19, 21, 27). Another major source of NTM may be contaminated

feed and water (5, 18, 22, 29, 30), peat and kaolin fed as supplements (17, 20, 27, 28, 29, 30), and soil in pens (14, 18). Mycobacteria may spread through various invertebrate species (15), including earthworms (11), dipterous insects (7, 8, 9, 10, 13, 16), cockroaches (11) and beetles (12, 13).

In industrialised countries, where the incidence of tuberculosis has decreased or stabilised, rates of NTM associated with lung infections now exceed those of tuberculosis. In African countries, where the prevalence of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) are high, recent studies have indicated that NTM may play a much larger role in tuberculosis-like disease than previously assumed (2). Therefore, the identification of the mycobacteria responsible for a disease and the discrimination of environmental from pathogenic species are relevant diagnostic issues that have important ramifications for the treatment of patients (24, 31). Knowing that identifying numerous species of mycobacteria through classical biochemical methods is time-consuming and error prone, the introduction of molecular biological methods which greatly improve the speed and accuracy of the process becomes imperative (25). Therefore, it is important to explore modern molecular biological methods which will provide accurate identification of these organisms. GenoType® Mycobacterium CM (Common Mycobacteria) assay (Hain test) has become an invaluable molecular method for differentiating members of the mycobacteria species.

In the past, several cases of tuberculous-like lesions have been observed in cattle and pigs during post-mortem inspections in Nigerian abattoirs; however, efforts have only been directed at identifying the incriminating agents in cattle (3, 4), with none documented in pigs. Because of this information gap and the public health implications of mycobacteria, the authors sought to confirm and characterise the species of mycobacteria responsible for tuberculous-like lesions in slaughtered pigs in a local abattoir in Ibadan, south-western Nigeria.

Materials and methods

Study site

The study was conducted in Bodija Municipal Abattoir located in Ibadan, south-western Nigeria. Ibadan lies between 7° 32´ N and 3° 54´ E; it is cosmopolitan in nature and is the biggest city in Nigeria. The abattoir is located in Ibadan North Local Government Area, one of the 10 local governments in Ibadan; it is the biggest in the state and serves as a major source of meat and pork, as well as other animal products, to the neighbouring areas in Ibadan city.

Duration of the study and population of animals screened

Samples were collected over a period of four months between March and June 2005, during which time 516 pigs of different breeds and ages (out of a total of 1,440 slaughtered during this period) were inspected for gross lesions suggestive of tuberculosis (Table I).

Post-mortem examination

This involved detailed examination of the submaxillary, cervical, hepatic, bronchial, mediastinal and mesenteric lymph nodes and other parenchymatous organs; including the lungs, liver and spleen. These were palpated and incised for evidence of tuberculous-like lesions. These lesions were collected, frozen at –4°C (Table I) and later cultured.

Detection of mycobacteria

The method used to process the lesions was based on a method previously described by Cadmus *et al.* (3). Firstly, the fat from each of the fresh specimens was trimmed away and the tissues were ground in a pestle and mortar with the addition of sterile distilled water. Equal amounts (5 ml) of

Table I
Cases of granulomatous lesions in slaughtered pigs between March and June 2005 in Bodija municipal abattoir, Ibadan, Nigeria

Month	Number of pigs slaughtered	Number of pigs examined	Lesions collected	Number of pigs with suspected TB lesions	Number of pigs confirmed positive for mycobacteria by culture
March	426	133	–	–	–
April	485	196	Lymph nodes, spleen	10	8
May	302	107	Lymph nodes	5	3
June	227	80	Lymph nodes, attachment of nodules to the spine	3	1
Total	**1,440**	**516**		**18**	**12**

TB: tuberculosis

specimen and activated N-acetyl-L-cysteine-sodium hydroxide were added to a sterile 50 ml centrifuge tube. The tube was vortexed until the specimen was liquefied. The mixture was allowed to stand at room temperature for 15 min with occasional gentle shaking. Prepared phosphate buffer was added to the 15 ml mark on the centrifuge tube and mixed, followed by centrifugation at 3,000 *g* for 15 min to 20 min. The supernatant was decanted away into a container with 3% lysol; 2 ml of phosphate buffer (pH 6.8) was added to re-suspend the pellet. The suspension was inoculated onto Middlebrook 7H11 slopes and Lowenstein Jensen slopes with pyruvate and/or glycerol, and incubated at 37°C for between 8 and 12 weeks.

Identification of mycobacteria isolates

Cultures were read on a daily basis in the first seven days and later every week, taking into consideration the colony morphology. Acid-fast bacilli (AFB) were detected using the Ziehl Neelsen method on all colonies that grew on 7H11 slopes.

Molecular typing

All AFB positive isolates were harvested for molecular typing analysis by scraping the growth from a slope into 200 µl of sterile distilled water and heating at 80°C for 1 h. The DNA strip assay (GenoType® Mycobacterium CM assay; Hain Lifescience, Nehren, Germany) was performed as recommended by the manufacturer. Briefly, for amplification, 35 µl of a primer nucleotide mixture (provided with the kit), amplification buffer containing 5 µl 10x and 2 µl Mm $MgCl_2$ and 0.2 µl DNA *Taq* polymerase (Biogene, United Kingdom), 2.8 µl RNAse free water (Promega), and 5 µl DNA in a final volume of 50 µl were used. The amplification protocol consisted of 15 min of denaturation at 96°C, followed by 10 cycles comprising 30 s at 95°C and 120 s at 58°C, an additional 20 cycles comprising 25 s at 95°C, 40 s at 53°C and 40 s at 70°C and final extension at 70°C for 8 min. Hybridisation and detection were performed using the recommended TwinCubator machine. This programme was initiated after mixing 20 µl of the amplification products with 20 µl of denaturing reagent (provided with the kit) for 5 min in separate troughs of a plastic well. One millilitre of prewarmed hybridisation buffer was added, then the membrane strips were placed into each trough. The hybridisation procedure was performed at 45°C for 30 min, followed by two washing steps. For colorimetric detection of hybridised amplicons, streptavidin conjugated with alkaline phosphatase and substrate buffer were added. After final washing, strips were dried with adsorbent paper.

The species isolated was determined by referring to the supplied interpretation chart. A band present on the strip correlates to the reaction zones which are specific for different probes.

Results

From the 516 animals inspected, 3.48% (18/516) had lesions suggestive of tuberculosis in different lymph nodes and organs. Culture results from these suspected tuberculous animals showed that 66.67% (12/18) of them had positive growth (Table I) within the first week. The results of the GenoType® Mycobacterium CM assay are shown in Table II. In 5 cases the assay failed to detect mycobacteria, in 3 cases it detected unspecified mycobacteria and in 4 cases it identified the mycobacteria as *M. fortuitum*. The four samples (i.e. P1, P2, P7 and P11) identified as *M. fortuitum* had bands visible for probes 7 and 14 on each strip (Fig. 1). The conjugate control, universal control and genus control were present for all samples, with the exception of genus control for sample P11.

Table II
Molecular typing of mycobacteria isolated from tuberculous-like lesions in pigs

Animal identification	Lesions collected	Culture result	Molecular typing
P1	Lymph node	+	*M. fortuitum*
P2	Lymph node	+	*M. fortuitum*
P3	Lymph node	+	Failed
P4	Lymph node	+	Failed
P5	Lymph node	+	Unspecified mycobacteria
P6	Lymph node, attachments to the spine	+	Unspecified mycobacteria
P7	Lymph node, spleen	+	*M. fortuitum*
P8	Lymph node	+	Failed
P9	Lymph node	+	Unspecified mycobacteria
P10	Lymph node	+	Failed
P11	Lymph node	+	*M. fortuitum*
P12	Lymph node	+	Failed

Discussion

The results confirmed the isolation of *M. fortuitum* as the causative agent of the tuberculous-like lesions seen in some of the slaughtered pigs. This was also re-emphasised by the short duration of the culture, which was within one week, and their smooth colony morphology. The discriminative

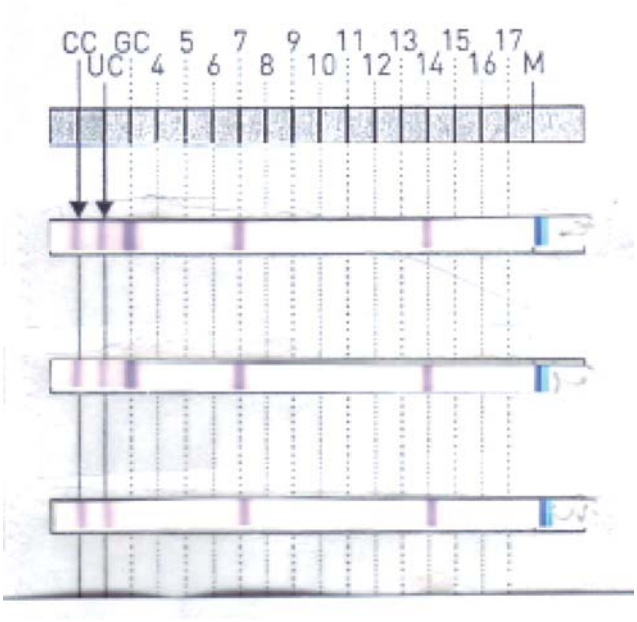

Fig. 1
GenoType® MTBC assay result of three of the pig isolates confirmed positive as *Mycobacterium fortuitum*

ability of the GenoType® assay to differentiate organisms belonging to the genus *Mycobacterium*, which has also been noted by Richter *et al.* (25, 26), provides a very good basis for the confirmation of *M. fortuitum* in these animals. The inability of the test to identify three of the isolates (classified as unspecified mycobacteria) further demonstrates the need for the inclusion of a second test (termed additional species [AS] on the GenoType® assay) which, incidentally, was not used in the present study. This test identifies species that are found more infrequently, such as *M. simiae, M. mucogenicum* and *M. celatum*. Thus, serial use of the two strips would have helped in identification of a larger spectrum of the mycobacteria classified as unspecified in the infected animals. Regarding the three unspecified mycobacteria, P5 had bands visible for probes 5, 6, 7, 10 and 14, which are not recognised on the CM kit. P9 had bands visible for probes 7, 10, and 14, which again are not recognised by the CM kit, and P6 had band 10 present, which may be further identified by using the AS kit. However, to confirm these other species, it would be necessary to use the GenoType® AS kit. This would be expensive, but would be worth exploring for further studies.

Findings from this study are similar to those established by Pavlik *et al.* (22), who linked the isolation of *M. fortuitum* to certain external environmental conditions. Contaminated water has been confirmed not only to be a good transport medium, but the main reservoir for the majority of NTM. In Ibadan, where water supply systems on farms are poor, most farmers resort to unchlorinated water sources and sometimes store water in reservoir tanks which may be contaminated with NTM since mycobacteria tolerate wide pH and temperature ranges (6, 23). Again, most of these pigs are kept on contaminated bedding which consists of wood shavings, sawdust, shredded paper and other wood materials that are known to be significant sources of *M. fortuitum* for pigs (15, 19, 21, 27). Another known source of *M. fortuitum* which these pigs have been exposed to is contaminated feed supply (22), which has been confirmed to be responsible for the spread of this pathogen. Farmers in Ibadan feed their pigs leftover foods and industrial waste, and, occasionally, the pigs are even allowed to graze on dump sites, which are good sources for contaminated food items. Moreover, pest control is not routinely carried out in most piggeries in Ibadan and the problem of insect/fly disturbance is widespread; consequently, pigs can become infected because *M. fortuitum* can easily spread through the various invertebrate species (11, 15) – including dipterous insects (7, 8, 9, 10, 13) and cockroaches (11) – that are commonly found in piggeries.

The above risk factors are major environmental issues which most of the pigs slaughtered in Nigerian abattoirs are exposed to. It follows, therefore, that the livestock attendants, butchers, meat processors and veterinarians who handle these animals are also exposed to the risk of infection. Such exposure may have been the cause of the *M. fortuitum* infection reported in an eight-year-old boy with ocular tuberculosis and a fourteen-year-old boy with osteomyelitis in Ibadan (1). These two human cases are important since, in some cases, young children help out in piggeries with poor environmental and hygienic standards. The correlation of hygiene standards and risk of infection is obvious from the findings of Pavlik *et al.* (22), who noticed a remarkable decline in pig infection due to *M. fortuitum* and other NTM after a consistent improvement in the hygienic conditions of pigs reared and slaughtered in a region of the Czech Republic. Of major importance is the co-infection of *M. fortuitum* and other NTM with HIV and AIDS, both of which are on the increase in Nigeria (32) among all groups, including the working population. It is extremely important, therefore, that environmental conditions in piggeries improve so that livestock workers are not exposed to these pathogens.

Conclusions

In conclusion, therefore, *M. fortuitum* should be considered to be a possible cause of any tuberculous-like lesions found in slaughtered pigs during post-mortem inspections in Nigeria. However, given the public health importance of this problem, further work in the area of molecular characterisation of the causes of tuberculous and tuberculous-like lesions in the larger pig population of the

country is required. Again, there is a need to improve the water and feed supplies of the pigs reared in the country as well as the management and hygienic conditions they are exposed to. Finally, since *M. fortuitum* is an opportunistic infection in HIV and AIDS patients, livestock workers with such health problems should take extra precautions to ensure that they work in hygienic conditions that do not expose them to environmental mycobacterial infections.

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Détection de *Mycobacterium fortuitum* dans des lésions de porcs abattus à Ibadan, Nigeria

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Résumé

Des lésions tuberculeuses ayant été trouvées chez des porcs abattus dans un abattoir municipal d'Ibadan (sud-ouest du Nigeria), une série d'inspections a été conduite pendant une période de quatre mois, au cours de laquelle 516 porcs ont été examinés, dont 18 présentaient à l'examen post-mortem des lésions macroscopiques évocatrices de tuberculose. L'identification de l'espèce de mycobactérie responsable de ces lésions a été réalisée par culture et confirmée au moyen d'une méthode de caractérisation moléculaire (épreuve GenoType® Mycobacterium CM [Common Mycobacteria]).

Les résultats montrent que 2,3 % (12/516) des animaux testés étaient infectés ; la présence de *Mycobacterium fortuitum* a été confirmée dans 33,3 % (4/12) de ces cas.

À la connaissance des auteurs, il s'agit du premier isolement confirmé de *M. fortuitum* chez des porcs abattus au Nigeria. Les mesures de prévention et de lutte devront être renforcées afin de limiter les sources potentielles d'infection mycobactérienne dans les élevages porcins. Ces infections présentent également un risque pour la santé publique, en particulier pour les travailleurs du secteur porcin.

Mots-clés

Épreuve GenoType® Mycobacterium CM – Ibadan – Inspection des viandes – Mycobactérie – *Mycobacterium fortuitum* – Nigeria – Porc – Santé publique – Technique moléculaire.

Presencia de *Mycobacterium fortuitum* en lesiones de cerdos sacrificados en Ibadan (Nigeria)

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Resumen

A fin de esclarecer la causa de lesiones tuberculosas en cerdos sacrificados en un matadero local de Ibadan (región Sudoccidental de Nigeria), se examinaron durante cuatro meses un total de 516 animales, en 18 de los cuales se observaron, en el curso del examen post-mortem, lesiones visibles que hacían pensar en una tuberculosis. Para identificar la especie responsable de las lesiones y confirmar su presencia se procedió a un cultivo micobacteriano y a una tipificación molecular (análisis genotípico GenoType® Mycobacterium CM [Common Mycobacteria]).

Los resultados pusieron de manifiesto que un 2,3% (12/516) de los animales examinados estaban infectados por micobacterias. En el 33,3% (4/12) de esos casos, el microorganismo en cuestión era *Mycobacterium fortuitum*.

Hasta donde saben los autores, esta es la primera vez que se confirma el aislamiento de *M. fortuitum* en cerdos sacrificados en Nigeria. Se impone ahora mejorar las indispensables medidas de prevención y control para reducir las posibles fuentes de infección micobacteriana en las piaras de cerdos reproductores. Estas infecciones también pueden tener consecuencias de salud pública, especialmente al afectar a los trabajadores de la industria porcina.

Palabras clave

Análisis GenoType® Mycobacterium CM – Ibadan – Inspección cárnica – Micobacteria – *Mycobacterium fortuitum* – Nigeria – Porcino – Salud pública – Técnica molecular.

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