Rev. sci. tech. Off. int. Epiz., 2005, **24** (3), 973-979

Abortion due to *Brucella abortus* in sheep in Nigeria

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Summary
This paper reports on a sporadic, naturally acquired infection of sheep with *Brucella abortus* on a privately owned farm in Toro near Bauchi, Nigeria. The abortions, which occurred in a flock of 28 Yankassa sheep, involved five ewes at the third month of gestation. Serum and milk samples from the flock were examined for *Brucella* antibodies by the Rose Bengal plate test, serum agglutination test (SAT) and milk ring test (MRT). The proportion shown as positive by SAT was 14.3%. All the five milk samples examined by MRT were positive. A total of seven isolates of *Brucella* were obtained from three milk samples and four vaginal swabs collected from aborting ewes. All isolates were identified and biotyped as *B. abortus* biovar 1. This biovar was also isolated from cattle maintained on the farm in association with the sheep.
The infection was attributed to the animal husbandry practices employed on the farm.

Keywords
Abortion – Brucella abortus – Brucellosis – Nigeria – Sheep.

Introduction

Brucellosis is an infectious disease of animals that is caused by a number of host-adapted species of the Gram-negative intracellular bacteria of the genus *Brucella*. The disease is characterised by abortion, retained placenta, orchitis and epididymitis (1). It is a worldwide zoonotic disease that is recognised as a major cause of heavy economic losses to the livestock industry, and also poses serious human health hazards (36). While the disease has been eradicated in most industrialised regions, its occurrence is increasing in developing countries (34). Brucellosis is widespread in Africa, where it remains one of the most important zoonotic diseases (23).

Brucellosis is endemic in Nigeria and, as elsewhere, causes severe economic losses to livestock farmers and ranchers, and is a serious risk to human health (2, 33).

Studies in various parts of the country indicate that the disease is widespread among cattle populations, particularly in ranches, livestock breeding centres and dairy farms. In these locations, the prevalence of the disease in cattle ranges between 3.7% and 48.8% (13, 14, 15, 16), while in the traditional nomadic Fulani cattle herds the prevalence is between 0.4% and 26% (6, 26, 27, 32). All these figures are based on serological surveys; there are few available reports based on the isolation of *Brucella* from cattle (6, 17, 29).

Field experience in Nigeria has shown that abortion is common in cattle, sheep and goats that are usually herded together, although the causes of such abortions have not always been investigated in detail in the laboratory (31). Brucellosis in sheep and goats is usually caused by *B. melitensis*. Infection with *B. abortus* is rare, although the association of *B. abortus* with abortion in sheep has been

demonstrated in several countries through isolation of the organisms (3, 4, 8, 24, 37).

In Nigeria, serological surveys for *Brucella* antibodies in sheep indicate a prevalence of 1.4% to 14.5% (7, 20, 30, 31). Little effort has been made to isolate *Brucella* from cases of abortion in sheep. The only available report in literature of the isolation of *Brucella* from such cases in Nigeria was by Okoh (31), who used milk from nursing ewes; the report did not indicate the biovar involved.

The aim of this paper is to describe an investigation of an abortion storm in a sheep herd, in which *B. abortus* biovar 1 was cultured from milk and vaginal swabs obtained from pregnant and aborting ewes.

Materials and methods

Description of the farm and animal husbandry

The farm

The farm involved in this study is located at Toro, about 90 km southwest of Bauchi, Bauchi State, Nigeria. It occupies about 250 hectares, half of which is used for farm buildings and the rest as grazing land for animals. The privately owned farm is essentially a poultry enterprise, with about 60,000 layers, but accommodates cattle and sheep on the premises.

The sheep flock

The flock consists of a total of 28 sheep of the Yankassa breed. The population structure is indicated in Table I. The initial stock consisted of four adult ewes and one ram purchased from a local market and introduced onto the farm in January 2001. The sheep were maintained under a semi-intensive husbandry system, fed mainly on concentrates such as corn mash, crushed millet and cotton seed cake, but obtaining part of their roughage by grazing on open grassland shared with the cattle on the farm. The ewes were mated by the rams in the flock. The long-term plan was to increase the size of the flock, with adult rams being sold for meat during local festivals, and loaned to peasant farmers to upgrade the stocks of local sheep.

In late January 2003, an outbreak of abortion involving two ewes in the flock was reported. This was followed two months later by another incidence of abortion involving three ewes. In each case the abortions occurred as short, sharp 'storms' at the third month of gestation. No sheep had been introduced onto the farm since the establishment of the flock. The animals had no history of vaccination; sheep and goats are not vaccinated against brucellosis in Nigeria.

The cattle herd

The herd of 20 animals consists of 11 white Fulani, one Adamawa red, two Friesian and six Friesian-white Fulani crossbreeds. The initial stock of nine cows, purchased locally from Fulani herdsmen, was introduced onto the farm one year after the sheep flock was established. The animals were maintained in a housing facility about 200 metres from the sheep pens. The cows were serviced by two Friesian bulls purchased from another farm. None of the animals were tested before being introduced onto the farm. No incidence of abortion had been reported in the herd since it was established.

Table I
Population structure of the sheep flock and tests performed

Sex	Age	Number examined	Number positive by serological tests			Vaccination status	Number positive by *Brucella* culture
			RBPT	SAT	MRT		
Male	Adult	4	0	0	NA	Unknown	0
	Sub-adult	3	0	0	NA	Unknown	0
	Lambs	2	0	0	NA	Unknown	0
	Total male	9	0	0	NA		0
Female	Adult	10	4	4	5	Unknown	5
	Sub-adult	3	0	0	NA	Unknown	0
	Lambs	6	0	0	NA	Unknown	0
	Total female	19	4	4	NA		5
Total number of sheep		28					

MRT: milk ring test
NA: not applicable
RBPT: Rose Bengal plate test
SAT: serum agglutination test
Adult: nine months or older
Lambs: < six months old
Sub-adult: six to eight months old

Collection and handling of blood samples

Approximately 10 ml of venous blood was collected from each of the 28 sheep and 20 cattle in 15-ml vacutainer tubes. The blood samples were allowed to clot, and then centrifuged at 3,000 rpm for five minutes. Serum samples were decanted into 5-ml plastic bottles, stored at –20°C and tested within a week of collection. The serum samples were examined for *Brucella* antibodies using the Rose Bengal plate test (RBPT), followed by the standard serum agglutination test (SAT). The tests were performed as described by Alton *et al.* (5), using antigens obtained from the Veterinary Laboratories Agency, Weybridge, United Kingdom (UK). For the RBPT, any agglutination was regarded as positive, while in the SAT a serum dilution of 1:50 with 75% clearing was taken as the criterion.

A total of seven vaginal swabs and five milk samples were obtained from pregnant and aborting ewes, and five milk samples from the cows. The milk samples were examined by the milk ring test (MRT) as described by Alton *et al.* (5).

Culturing of milk and vaginal swabs

The culture samples collected from each aborting ewe are shown in Table II. It was not possible to isolate cultures from aborted foetuses and placentas as these materials had been discarded or buried by the time the investigation was conducted. Primary isolation of *Brucella* was made by culturing the samples on Farrell's modified serum dextrose agar (21) prepared from blood agar supplemented with 5% horse serum, 1% dextrose, and ready-mixed antibiotic supplement at the following amounts per ml of media: Bacitracin, 25 IU; Polymyxin B, 5 IU; Cycloheximide, 100 µg; Nalidixic acid, 5 µg; Nystatin 100 IU; and Vancomycin, 20 µg. The inoculated plates were incubated aerobically at 37°C in an atmosphere of 5% to 10% CO_2, and examined after three to five days for *Brucella*-like colonies. The plates were discarded if no growth was evident after seven to ten days of incubation.

Isolates obtained from culture samples were identified as described by Alton *et al.* (5). Representative strains were examined and subjected to full typing, using currently recommended procedures (5). The monospecific antisera and the *Brucella* phages used in this study were obtained from the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Collaborating Centre for Brucellosis Reference and Research, Veterinary Laboratories Agency, Weybridge, UK.

Table II
Isolation of *Brucella* from culture samples obtained from ewes

Physiological status	Culture sample	*Brucella* culture
Abortion	Vaginal swab, milk	*Brucella abortus* biovar 1
Abortion	Vaginal swab, milk	*Brucella abortus* biovar 1
Abortion	Milk	*Brucella abortus* biovar 1
Abortion	Vaginal swab	*Brucella abortus* biovar 1
Pregnant	Vaginal swab	*Brucella abortus* biovar 1

Results

Four of the 28 serum samples (14.3%) from the sheep investigated were shown as positive for *Brucella* antibodies by both the SAT and RBPT. Only one of the aborting ewes gave a positive reaction to both tests. Two out of 20 serum samples (10%) obtained from cattle were found to be positive by both the SAT and RBPT. All the five milk samples from sheep and two out of five from cattle tested positive by the MRT.

Seven isolates of *Brucella* were obtained from culture samples. Three were from milk samples, three from vaginal swabs of aborting ewes, and one from a vaginal swab of a pregnant ewe. *Brucella* was also cultured from two out of five milk samples obtained from the cows. All isolates were Gram-negative coccobacilli, which grew readily on Sabouraud's dextrose agar (SDA), producing colonies typical of *Brucella* species morphology after incubation at 37°C in 5% CO_2 and also in air.

All cultures produced H_2S and were strongly positive to the urease test, giving a positive reaction on Christensen's medium after 24 hours. They grew on SDA containing 20 µg/ml of fuschin, but not on thionin at 20 µg/ml.

All the strains were sensitive to lysis by Weybridge, Tbilisi and Berkeley phages at routine test dilutions. Agglutination tests with A and M monospecific antisera showed that the isolates were A antigen dominant. These properties were consistent with those of *B. abortus* biovar 1.

Discussion

The significantly high prevalence of *Brucella* antibodies, the clinical signs exhibited and the recovery of *B. abortus* from milk and vaginal swabs of the aborting sheep indicate that the reported abortion storm was due to *B. abortus*. Most surveys for brucellosis in Nigeria rely on serological tests only, without isolation of *Brucella*, and this can be misleading. Confirmatory diagnosis of brucellosis is provided by the isolation of *Brucella*. The successful isolation of *Brucella* from aborting animals, as described in this report, is therefore very significant.

The source of infection for these animals was not easily ascertained. The vaccination and brucellosis status of the

animals was not known; they were neither vaccinated nor tested for brucellosis when they arrived on the farm. The sheep share the same grazing ground as the dairy cattle. During the course of the investigation, *B. abortus* biovar 1 was isolated from two out of five milk samples obtained from the cattle, which – as previously mentioned – had been introduced onto the farm a year after the sheep flock was established. Prior to their introduction onto the farm, there had been no reported case of abortion in the sheep flock. It is probable that the sheep acquired *B. abortus* from the cattle on the farm, possibly from using a field that had been infected by the cattle.

Brucella abortus has been reported as a cause of abortion in sheep in Nigeria (31), but the report did not indicate the biovar involved. The isolation and biotyping of *B. abortus* biovar 1 from aborting sheep reported here provides further information. Furthermore, the report described here is one of only a few documented studies originating in Nigeria that provides direct evidence, by isolation and biotyping, that *B. abortus* is the causative agent of the disease in this country.

Previous reports indicate the isolation of *Brucella* from cattle (6, 17, 29), goats (19) and horses (28, 32) from various states of Nigeria, with varying results relating to the prevailing biovars of *Brucella*. These studies, together with the present report, do indicate that *B. abortus* biovar 1 may be the predominant strain associated with the disease problems in Nigerian livestock.

While a broad host range generally exists for *Brucella* species, *Brucella* infection follows a very strict, host-related hierarchy of pathogenicity (1). Thus, goats are the natural hosts of *B. melitensis* and sheep are preferred hosts of the pathogen. Humans are end hosts, as is indicated by Malta fever. Cattle are natural hosts of *B. abortus* and pigs are hosts for *B. suis*. Inappropriate management may allow the disease to be transferred to a heterologous host such as sheep. In such cases, the disease will cause only sporadic abortion (11), as is indicated by the present report. This study also demonstrates that *B. abortus* has crossed the border from its natural host in cattle and has been transmitted to sheep, leading to abortion in the latter species.

Brucella organisms were recovered from clinical samples obtained from five of the 28 sheep on the farm (17%). This helps to quantify the risk of the disease being perpetuated. Moreover, the five animals from which *Brucella* were isolated belonged to the same group of adult ewes, re-emphasising the risk associated with the breeding of those animals.

Serological tests seem to have misdiagnosed at least one of the animals from which *Brucella* was isolated. The results demonstrated that the animal was negative for both RBPT and SAT. Serological tests are used to detect antibodies in the serum, uterine discharge, vaginal mucus, milk or semen plasma of animals thought likely to have brucellosis (11). These body fluids may contain different quantities of antibodies of immunoglobulin (Ig) M, IgG1, IgG2 and A types directed against *Brucella* (10). It has been shown that the different serological tests used in the diagnosis of brucellosis vary considerably in their ability to detect antibodies of a particular immunoglobulin class (9). Infected animals may or may not produce all antibody isotypes in detectable quantities. Also, after the disease has entered the chronic stage, antibody titres may decline or remain around the diagnostic threshold (38). Therefore, since the capacity of serological tests to reliably detect brucellosis depends on antibodies that may or may not be present at the time of examination, some infected animals will inevitably elude detection (22), remaining bacteriologically positive but giving negative results in serological tests (12, 25, 38). This may be what happened in the present case.

The MRT was used for screening the milk samples obtained from the animals involved in this case. However, this test – which is widely used to detect brucellosis in cattle – is not sensitive or effective enough to detect the disease in sheep (35). The MRT is therefore not recommended for use on sheep (36).

Cattle, sheep and goats are the principal farm animals in Nigeria. One factor contributing to the spread of brucellosis in the country is the herding of these animals together, which is the normal practice of the traditional nomadic Fulani pastoralists. The Fulani are accustomed to an extensive system of management and manage about 95% of the total animal population in Nigeria (32). Such husbandry practices, with animals of different species being herded together, increase the likelihood of animals being exposed to the disease. This factor should be taken into consideration in the planning and execution of control programmes. Movements of animals should be controlled by appropriate legislation and regulations.

The spread and transmission of infection are made more likely by the trend towards more intensive animal production in the absence of either the required veterinary infrastructure or an appropriate level of socioeconomic development among the animal handlers (34). In the present case, the sheep and the cattle were not tested for brucellosis before they were introduced onto the farm, nor was veterinary support sought from the time the farm was established until the outbreak of abortion. This kind of procedure is among the factors that hamper livestock production in Nigeria. The authors suggest that animals should be serologically tested for brucellosis before they are introduced into farms.

Conclusions

Brucella abortus readily infects cattle and spreads among pregnant animals, and can cause abortion. This means that schemes for the control and eradication of bovine brucellosis where infected sheep and goats are present must include its eradication from these animals as well (31). The control programme, which should focus on a prophylaxis campaign for bovine brucellosis using the *B. abortus* B19 vaccine, should operate alongside a surveillance campaign for sheep and goats. At present, there is no official programme for the control of brucellosis in Nigeria. The authors suggest that a plan for a nationwide control programme for brucellosis in all animals (including small ruminants) should be established. This is particularly important for developing the unrealised economic potential of Nigeria's livestock industry, and in view of the health problems posed by the spread of the disease (18).

The report on the identification of *B. abortus* infection in sheep associated with an abortion storm, which also provides significant information on the pathogenicity of these organisms, fills an important gap in the literature on this disease and its implications deserve careful study.

Acknowledgements

The authors are grateful to Mrs J.A. Stack and Mrs L.L. Perrett of the FAO/WHO Collaborating Centre for Brucellosis Reference and Research, Veterinary Laboratories Agency, Weybridge, UK, for kindly supplying the antisera and *Brucella* phages. The authors also thank Dr A. Ogbe for referring the case to them.

Avortements dus à *Brucella abortus* chez des ovins au Nigeria

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Résumé

Le présent article décrit une infection sporadique à *Brucella abortus* naturellement contractée par les ovins dans une exploitation privée à Toro près de Bauchi, au Nigeria. Les avortements, qui ont été observés dans un troupeau de 28 moutons Yankassa, ont concerné cinq brebis au troisième mois de gestation. Les anticorps dirigés contre *Brucella* ont été recherchés dans les échantillons de sérum et de lait prélevés dans le troupeau en utilisant l'épreuve à l'antigène tamponné, le test de séro-agglutination (SAT) et l'épreuve de l'anneau (ring-test). Le test de séro-agglutination a donné 14,3 % de résultats positifs. Les cinq échantillons de lait ont tous donné à l'épreuve de l'anneau un résultat positif. Au total, sept souches de *Brucella* ont été obtenues à partir de trois prélèvements de lait et de quatre écouvillonnages vaginaux pratiqués sur des brebis qui avortaient. Toutes les souches ont été identifiées comme étant *B. abortus* de biotype 1. Ce biotype a également été isolé chez des bovins élevés avec les ovins dans l'exploitation.

L'infection a été attribuée au mode d'élevage du bétail pratiqué à la ferme.

Mots-clés

Avortement – Brucella abortus – Brucellose – Nigeria – Ovin.

Abortos provocados por *Brucella abortus* en ovejas de Nigeria

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Resumen

Los autores informan de un episodio de infección de ovejas por *Brucella abortus* en una explotación privada de Toro, cerca de Bauchi (Nigeria). Se trata de una infección esporádica y adquirida de forma natural. Los abortos, ocurridos en un rebaño de 28 ovejas Yankassa, afectaron a cinco hembras en su tercer mes de gestación. Tras extraer muestras de suero y leche, se sometieron éstas a pruebas de aglutinación en placa de rosa de Bengala, seroaglutinación y anillo del leche para detectar anticuerpos antibrucélicos. La prueba de seroaglutinación deparó un porcentaje de muestras positivas del 14,3%. También resultaron positivas las cinco muestras de leche analizadas con la prueba del anillo. A partir de tres muestras de leche y cuatro de torundas vaginales practicadas a las hembras con aborto se obtuvieron siete cultivos puros de brucelas, que tras la oportuna tipificación resultaron corresponder en su totalidad al biovar 1 de *B. abortus*. Este mismo biovar se aisló también en bovinos que convivían con las ovejas en la granja.

Se atribuyó la infección a los métodos de producción animal utilizados en la explotación.

Palabras clave

Aborto – Brucella abortus – Brucelosis – Nigeria – Oveja.

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