

Bacteriological and serological studies of bovine brucellosis on the Obudu Plateau, Cross River State, Nigeria

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ABSTRACT

Brucellosis is an important veterinary and public health problem worldwide especially in developing countries where the disease has remained a neglected zoonosis. Blood was collected from a total of 354 cattle including vagina swabs (14) and hygroma aspirate (1) from cows reported to have recently aborted and a bull with carpal hygroma respectively. Sera was harvested from the blood samples and screened for antibodies to Brucella using Rose Bengal Plate Test (RBPT) while the vagina swabs and hygroma aspirates were cultured for isolation of Brucella. Biotyping was carried based on biochemical activity and lysis of the isolates by Tbilisi (Tb), Weybrigde (Wb) and Berkely (Bk₂) Brucella bacteriophages. Out of the 354 sera samples 149 (42.09%) were positive for Brucella antibodies while only 2 samples; a vagina swab and a hygroma aspirate from different animals (a cows and a bull respectively) exhibited typical cultural characteristics of Brucella and were identified as Brucella abortus biotype 1. The findings of this study have provided for the first time, serological and bacteriological evidence suggesting the circulation of Brucella abortus biotype 1 among the pastoralist cattle population on the Obudu plateau. This finding represents a threat for the introduction and spread of the disease to other areas as well as other animal and human. Urgent institution of brucellosis control measures as well as proper enlightenment of the herdsmen and public on brucellosis and preventive measures are recommended.

Keywords: Abortion, *Brucella abortus* Biotype 1; bovine, Cross River; Hygroma, Isolation, Obudu, Nigeria, serological, vagina swab.

INTRODUCTION

Bovine brucellosis is an economically important disease in production animals as well as a public health threat because of its zoonotic potential worldwide [1]. It is majorly caused by *Brucella abortus* which has cattle as its natural host though it has been reported in other species of animals [2]. Bovine brucellosis is characterized clinically by impaired fertility specifically abortion, metritis, orchitis and epididymitis [3].

Brucellosis as a zoonosis causes a debilitating febrile illness in humans known as 'Mediterranean or undulant fever, which can be acquired as a result of contact with infected animals, contaminated materials or consumption of infected products such as meat, cheese or meat [4-6].

Although, data from serological surveys predominates as evidence for the endemicity of bovine brucellosis in Nigeria [7-13], a few bacteriological studies have also been documented [14-17]. Relying on serological evidences alone can be potentially misleading and confirmatory diagnosis which is based on cultural isolation or molecular identification of the organism from the infected host is time consuming, complex, and positive animals sometimes may yield negative culture results [15, 18].

Obudu plateau located at an altitude of about 1,716 meters above sea level has a semi- temperate mountainous climate that offers a total contrast and welcome respite from Africa's tropical heat with a temperature of between 4°C - 10°C and 26°C - 32°C from June – September and November – January respectively. This plateau harbours the renowned Obudu mountain resort (Obudu cattle Ranch) which attracts both local and international tourists. The ranch is home to about 30,000 pastoralist herd and 500 exotic dairy cattle belonging to the management of the Obudu mountain resort.

Brucellosis in the cattle population is therefore a serious threat not only to livestock production on the plateau but also to public health. This is because the visitors, tourist or herdsman during their activities may come in contact with contaminated environment, infected animals or consume contaminated products such as milk and cheese. However, to the best of our knowledge and after extensive literature search there is no documented evidence on the status of brucellosis on the Obudu plateau. Therefore, this study was conducted to determine the status of brucellosis among the nomadic herds on the Obudu plateau with specially emphasis on the cattle herd surrounding/located close to the Obudu mountain resort (Obudu cattle ranch).

MATERIALS AND METHODS

Study Area: Obudu plateau and home to the famous Obudu Mountain Resort (Obudu cattle Ranch) is located between latitude 6° 37'N and longitude 9° 20'E in Obanliku Local Government Area of Cross River state, Nigeria. It is an area of about 1,057 km² with a human population of about 110,320 [19]. Cross river state where Obudu is located is bounded by Benue, Ebonyi, Akwa-Ibom, Abia and Enugu states in Nigeria and Republic of Cameroon. The inhabitants are mostly farmers comprising of the Indigenous community and the Fulani (pastoralist) herdsman. Cattle production system in this area is mainly extensive except for the dairy animals kept by the management of the Obudu mountain resort and includes sedentary and transhumance/nomadic pastoralists.

Study Design: The study design used was a cross sectional. About 10-15% of the total population of cattle counted in each herd were randomly selected (with allowance made for animals that have recently aborted or showing signs of brucellosis such as hygroma) for sampling. Only animals greater than one year of age and herds for which consent was granted were included in the sampling.

Sampling: Blood samples were collected from a total of 354 cattle of different ages from the different herds on the plateau. In addition to the blood samples vagina swabs were collected from the 14 cows that were reported to have recently aborted and hygroma fluid from a bull with carpal hygroma. Serum was separated from the blood after clotting and used for serological studies. All procedures were carried out aseptically and samples were transported on ice to the *Brucella* Research Unit, National Veterinary Research Institute, Vom Plateau state where the vagina swab from the cows and hygroma aspirate from bull were used for bacteriological studies.

Cultural Isolation and Biotyping: This was carried out as described by Alton et al. [20] and reported elsewhere by Oholi et al. [15]. Briefly, each sample (vagina swab or hygroma aspirate) was inoculated onto Farrell's modified serum dextrose agar containing 5% calf serum, 1% dextrose, and ready mixed antibiotics supplemented at the following amount per ml of media: bacitracin (25 iu), polymyxin B (5 iu), cycloheximide (100 µg), nalidixic acid (95 µg), nystatin (100 iu) and vancomycin (20 µg). The plates were incubated at 37°C under aerobic and microaerophilic conditions and observed for *Brucella*-like colonies for 5-7 days. Plates showing no visible growth after 7-10 days were discarded. Colonies typical of *Brucella* species were sub-cultured on serum dextrose agar from which subsequent growth was subjected to Gram staining, modified Ziehl-Nelsen staining, catalase, and oxidase production.

The isolates were further subjected to slide agglutination with *Brucella* A and B monospecific antisera, detection of H₂S and urease production, dye sensitivity by growth in the presence of basic fuchsin (20 µg ml⁻¹), thionine (20 µg ml⁻¹) and susceptibility to lysis by Tbilisi (Tb), Weybrigde (Wb) and Berkely (Bk₂) *Brucella* phage [21] to enable

biotyping. The reference strains of the phages and antisera were obtained from the Veterinary Laboratory Agency (VLA), Weybridge, United Kingdom (UK).

Serological Test

Rose Bengal Plate Test (RBPT): Serum samples were screened using the Rose Bengal plate test (RBPT), employing stained *B. abortus* antigen (VLA, Weybridge, UK) and known positive and negative reference sera at the National Veterinary Research Institute (NVRI) Vom, Plateau state, Nigeria. Briefly, 30 μ l of test serum was placed on a white ceramic tile and the same volume (30 μ l) of the RBPT antigen was placed beside the test serum. The two were mixed thoroughly with a sterile applicator stick and rocked gently for 4 minutes. The mixture was then observed for agglutination. Samples that showed distinct agglutination were recorded as positive, while those with no sign of agglutination were recorded as negative.

RESULTS AND DISCUSSION

A total of 354 cattle sera were tested for *Brucella* antibodies using the Rose Bengal plate test (RBPT), out of which 149 (14.09%) were positive reactors to *Brucella* antibodies (Table 1 and Table 2).

One hundred and thirteen of the sera tested were from males while 241 were from females out of which 40 (35.40%) and 109 (45.23%) were positive for *Brucella* antibodies respectively. Of the 354 cattle sampled and tested 194 were > 5 years of age out of which 10 (20.00%) were positive for *Brucella* antibodies. Also 110 of the cattle were within the 2 – 5 years age group while 50 were < 2 years out of which 44 (40.00%), and 95 (48.97%) were also positive for *Brucella* antibodies using the RBPT (Table 1).

Though, Friesian breed comprised majority (131) of the breed sampled compared to Kuri breed (117) and White Fulani breeds (106), Kuri breed had the highest number: 73 (62.39%) of cattle positive for *Brucella* antibodies. Only 10 (7.63%) Friesians and 66 (62.26%) of the White Fulani breeds were positive for *Brucella* antibodies (Table 1).

There was no statistically significant association between positivity for *Brucella* antibodies and sex of the cattle. However, there was a statistically significant association between positivity and age of the cattle as well as the breed of cattle (Table 1).

Table 1: Sex, age and breed specific prevalence of bovine brucellosis from herds on the Obudu plateau, Cross River State, Nigeria using Rose Bengal plate test (RBPT)

Variables	Total no. Sampled and Tested	Total no. Positive (%)	χ^2 (p value)
Sex			
Males	113	40 (35.40)	3.05 (0.0807)
Females	241	109 (45.23)	
Age			
< 2	50	10 (20.00)	13.97 (0.0009)
2-5	110	44 (40.00)	
>5	194	95 (48.97)	
Breeds			
Kuri	117	73 (62.39)	101.3 (0.0001)
Friesian	131	10 (7.63)	
White Fulani	106	66 (62.26)	

All fifteen animals from whom samples were collected for cultural isolation were sero-positive for *Brucella* antibodies by RBPT. However, growth and staining characteristics typical of *Brucella* were observed in only two out of the fifteen culture samples; one from vagina swabs and the other from the hygroma aspirate (Table 2). The isolates showed agglutination with *Brucella* A monospecific antiserum and grew in the presence of basic fuschin but not thionine. Lysis of the isolates by Tb, Wb and Bk₂ *Brucella* bacteriophages was observed indicating the isolate was *Brucella abortus* biotype 1.

Table 2: Prevalence and distribution of bovine brucellosis from different samples on the Obudu plateau, Cross River State, Nigeria

Variables	Total no. Sampled and Tested	Total no. Positive (%)
Vagina swabs (cultural isolation)	14	1 (7.14)
Hygroma fluid (cultural isolation)	1	1 (100)
Sera (screened using RBPT)	354	149 (42.09)

Brucellosis is classified as one of the neglected zoonoses worldwide [22]. Endemicity of bovine brucellosis has been established at different times from various regions and management systems in Nigeria [9-10, 12-13]. The results of this study further support earlier findings that *Brucella abortus* biotypes 1, 2, 3 and 4 (with biotype 1 being the

commonest) are the endemic biotypes [15-17, 23-26]. Also of recent *Brucella abortus* biotype 1 was isolated from cattle with hygroma in Benue state which shares boundary with Obudu [26]. Biotypes 1, 3, 6, 7, 8 and 9 have also been reported around Africa [27-31] with a predominance of biotypes 1 and 3 in countries of West Africa where Nigeria is located [30-32]. The occurrence of brucellosis in this cattle population poses an economic threat (to the immediate livestock owners due to productivity losses) as well as to animal health because of the potential of spread to other livestock around especially as this area provides a large space, pasture for grazing and conducive climatic condition for congregation of livestock during the year. Also of note is the close contact that exists between these herdsmen and their livestock as well as the consumption of unpasteurized milk directly from the cows which may serve as a potential source of human infection.

There is an uncontrolled movement of livestock within and into Nigeria coupled with the breakdown of disease surveillance and quarantine services [33]. The herdsmen are involved in the practice of moving their animals around in search of pasture depending on the season. This practice brings the animals in contact with animals from other herds at the migratory routes, grazing and watering points resulting spread of diseases like brucellosis. Brucellosis has also been reported in neighbouring states; Benue [26], African countries that share boundary with the cattle ranch; Cameroon, 8.4% [32] and where some of the pastoralists migrate to in search of pastures; Chad 7% [34], Togo 41% [35], Sudan 2% [36], Ivory Coast 10.3% [37] and Ghana 6.6% [38]. These may be likely sources for introduction of the disease into the cattle population. Other likely sources of the infection include some practices common among the herdsmen such as sharing of breeding bulls between herds which may result in venereal transmission of brucellosis [13], purchasing of infected livestock from markets and introducing them into the herd without quarantine [14] and interaction of cattle during their migration with wildlife [39]. The role of wildlife as a source of infectious agents especially brucellosis and tuberculosis to cattle has also been highlighted as an emerging threat [39-41].

Hygromas, usually involving the carpal or tarsal joints, are a common manifestation of brucellosis in some tropical countries and may sometimes be the only obvious indicator of infection [42]. The *Brucella* organism localizes in the joints producing inflammation and subsequently fluid accumulation and visible swelling (hygroma). This fluid contains large number of the organism which may be the reason we were able to isolate the organism from the aspirate. Majority of the pastoralist however, usually indulge in a practice called "firing", where a red hot knife is placed on such hygroma aimed at incising it to release the accumulated fluid and in the process releasing large numbers of the organism in the fluid. This contaminates the environment resulting in spread of the disease.

Although bovine brucellosis eradication plans have been in place for many decades in Nigeria due to reports of its endemicity, the level of infection in bovine herds is still very high as a result of non implementation. This non implementation is mostly due to the absence of political will by the government and corruption when it comes to payment of compensation.

CONCLUSION

In conclusion, the results of the present study have revealed via serological and bacteriological methods that bovine brucellosis is present among cattle on the Obudu plateau. *Brucella abortus* biotype 1 was isolated from the aborting cows and hygroma fluid and may be the circulating biotype in this area. These findings suggest the area may be a *Brucella abortus* foci and serving as source of infection and spread of the disease to unaffected animals around and those on the organized dairy farm owned by the resort. The unsuspecting human population (herdsmen, visitors and tourist) may be at risk of infection when they come in contact with infected animals or consume contaminated products during their activities. There is an urgent need for political will by the government of Nigeria for the implementation of brucellosis control measures and for more public health enlightenment programmes aimed at developing proper awareness on preventive practices such as proper pasteurization of milk, hygienic practices and good husbandry practices. There is need for a large scale study aimed at determining the extent of brucellosis among the cattle and human population in the area given the close contact that exists between these herdsmen and their animals.

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