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Seroprevalence of brucellosis in donkeys (*Equus asinus*) and assessment of donkey management practices in Gamawa local government area, Bauchi state, Nigeria

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Target Audience*: Researchers, farmers, herders and policy makers.*

Abstract

This study was conducted to determine the seroprevalence of brucellosis in donkeys (Equus asinus) and its association with donkey management practices in Gamawa Local Government Area, Bauchi State, Nigeria using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT). A total of 1000 donkeys were sampled comprising of 585 males and 415 females. Two hundred donkeys were sampled from each of the five clusters in the study area viz: Gamawa East, Gamawa West, Udubo East, Udubo West and donkey Market. Simple percentages and chi-square (χ^2) test were used to analyzed the data by subjecting it to SPSS-16 statistical software. The overall prevalence of brucellosis in donkeys was 114 (11.4%), out of which 51 (8.7%) males and 63 (15.2%) females were positive. Prevalence of Brucellosis was higher in females 63 (15.2%) than males. Breed of donkeys has significant influence on prevalence of brucellosis. Prevalence was higher among ≥7 age group 16 (19.8%) than in 5-6, 3-4 and ≤2 age groups ($P<0.01$) and in donkey market 34 (17%) than the other locations ($P<0.05$). It can be concluded from the findings of this study that there is 11.4% prevalence of brucellosis among donkeys in the study area with higher prevalence in female donkeys, older donkeys and donkey market.

Key words: *Seroprevalence, Brucellosis, Donkey, (RBPT), (SAT), Gamawa.*

Description of Problem

Donkeys are in everyday sight throughout most of Northern Nigeria, little is documented of their diseases, breeds, distribution and productivity under traditional management. According to the Food and Agriculture Organization, the global donkey population is estimated at 44 million. The donkey's role depends on the communities in which it lives and works. Usually, its traditional role includes riding, ploughing and carting, with fewer roles in entertainment and food production (1).

As compared to other domesticated species, donkeys have received little or no attention from development agencies despite the fact that they are essential to the subsistence strategies of many communities in semi-arid regions (2). Donkeys can provide power for a wide variety of rural and urban tasks and appear to stay healthy on varied and often poor-quality diets and with only modest

management inputs. The great majority of donkeys in the world (probably over 95%) are kept specifically for work. Their most common role is for transport, pack transport or pulling carts. They may also be used for farm tillage (3). In certain countries they may assist in threshing, pulling water, milling or other operations (3). Donkeys are not conventional source of meat. Interest in donkey milk has recently increased, especially in Europe, as it represents an alternative food in cases of bovine milk proteins allergy and in the prevention of metabolic pathologies (4). Donkey milk has a somewhat similar chemical composition to human milk (5). Recently, the growing importance of milk production from species other than cattle has been also highlighted in tackling the growing global demand for milk from donkey could be explore to meet the growing global demand as well as solving problem of low animal protein (milk) intake in under developed countries and sub-Saharan African. However, Brucellosis remains a daunting challenge. *Brucell*osis in donkeys is important not only as clinical existence but also as a source of infection to the general public (6).

Brucellosis is an important zoonotic disease worldwide causing serious human health problems and substantial economic losses for the livestock industry (7). The disease is highly contagious in domestic and wild animals and is one of the most important and widespread zoonosis in the world (8). Though it has been eradicated in many developed countries like Europe and America, Australia, Canada, Israel, Japan and New Zealand (9), it remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (10, 11). Although prevalence is high and variable in many countries, surveillance for the disease is generally poor. Factors assumed to be responsible for variation in prevalence include purchase of infected animal from the market for replacement or upgrading, nature of animal production, demographic factors, regulatory issues, climate, deforestation and wildlife interaction. Furthermore, one major factor contributing to the spread of the disease is the free movement of animals practiced by the nomadic Fulani herdsmen, who own about 95% of all food animal populations in Nigeria. Other factors that may influence the prevalence of brucellosis in Nigeria include management system, the herding of different species of animals together, use of common pastures and water sources, age, breed, sex, lactation status and season. However, other variables such as pregnancy status and state have not been assessed. All these risk factors need to be taken into consideration in designing and execution of effective control programmes in Nigeria.

Considering the damage done by the infection in animals in terms of decreased milk production, weight loss, infertility, abortion, stillbirth, birth of weak offspring's, retained placenta and lameness. Death may occur as a result of acute metritis, followed by retained foetal membranes (12). Therefore, there is need for knowledge, public awareness and improved management. Infection occurs via contaminated feed or water, by inhalation, through the conjunctiva, or by contact with infected aborted materials; while calves become infected in utero or via infected colostrum or milk. Venereal transmission has also been reported. In fully susceptible herds, abortion rates vary from 30 to 70%. Infection may be life long, and during subsequent pregnancies there is invasion of the gravid uterus and allanto-chorion; abortion rarely recurs, but uterine and mammary infection recurs (12). Since the reproductive performance of these carrier animals is unaffected, they are retained in herds in Nigeria despite the presence of pathognomonic clinical signs in some cases, making effective

control programmes extremely difficult. Transmission from infected animal to human can either be direct through contact with infected material, or indirect through consumption of animal products. Signs and symptoms of *B. canis* infections in humans are generally similar to those of brucellosis caused by *B. abortus* and *B. melitensis*. Manifestations are frequently non-specific, and may include; fever (often periodic and nocturnal), fatigue, headache, weakness, malaise, chills, sweats, weight loss, hepatomegaly, splenomegaly, and lymphadenopathy.

Materials and Methods

Experimental location

This research study was conducted in Gamawa Local Government Area of Bauchi State, Nigeria (North Eastern Nigeria) bordering Yobe State to the East and Jigawa State to the West. It lies between latitude 12°08'12"N of the equator and longitude 10°32'23"E of the Greenwich meridian. Headquarters is in Gamawa town. It has an area of 2,925 km^2 and a population of 286,388 as at 2006 census. The annual rainfall ranges between 700mm to 1600mm which starts in May and ends in October. The wettest months are from July to September and dry season commences fully in November and ends in April. The relative humidity ranges between 20-30% and the mean monthly temperature ranges between 26°C to 39°C. It is located in the Sub-Sudan and Northern Guinea Savannah Zone. The predominant ethnic group in the area are the Hausa, Template, Fulani people, Fulfulde with the kare kare people living in the East (13).

Experimental animals

A total of 1000 sera samples were taken from Donkeys reared traditionally in the study area which comprised mainly of four breeds classify according to their coat colour namely; Auroki (rust to red), Duni (dark brown to black), Fari (pale cream to white) and Idabori (grey to light medium brown) breeds. The total number of the study animals was obtained using a combination of random and convenient sampling (14) from every herds/household that was accessible in the study area.

Sample size determination

The calculation for the sample size was based on the multistage random sampling formular, $n= Z^2 Pexp(1-P exp) /L^2$ as described by (15), where, Z= confidence level given as 1.96, L= desired absolute precision (±5%) and P exp.= expected prevalence (20%). Although the actual sample size was found to be approximately 246 donkeys based on the calculations. One thousand donkeys (1000) across all breed, age and sex were selected from 68 herds/households in order to increase the accuracy and reliability of the test.

Experimental design

Sampling was done by combination of convenience sampling and random sampling methods (15) in which five wards where donkeys are kept were identified and the sampling sites were selected based on the availability of donkeys. Verbal approval was sought from the Village Head (*Hakimi*) of Gamawa who assigned one of his aides (*Magatakarda*) to assist in reaching the donkey owners. Gamawa East, Gamawa West, Udubo East, Udubo West and donkey market were considered as clusters. In each cluster, both male and female donkeys were selected. Two hundred (200) donkeys were selected in each of the clusters. Data collected includes; sex of donkey, breed of donkey, location of donkey and management practices (Herd size, management system, source of drinking water and coming together of donkeys with other species).

The study was conducted using serological test and questionnaire survey. Blood collection and administration of the questionnaire were carried out from March-

September, 2016. The questionnaire was designed to provide information on sex of donkey, breed of donkey, age of donkey, location of donkey, herd size, management practice, source of drinking water and coming together of donkey with other species and it was administered by random selection of the respondent through verbal interview at herd/household level.

Blood collection and laboratory analysis

Following proper restraint of donkeys, five millilitres (5ml) of blood was aseptically collected via the jugular vein, using hypodermic syringe and needle. The blood collection was done 6.00am-10am daily. Blood in the syringe was placed in a slanted position for one hour under shade to get the serum separated from the clotted blood. Serum samples were placed in cool box containing ice packs and stored in a freezer at -20^{o} C before serological analysis. Serological tests were conducted in the Abubakar Tafawa Balewa University laboratory using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) [both from Onderstepoort Biological Products Ltd, South Africa] as screening and standard test for brucellosis respectively according to (16) and (17). The parameters that were measured in both RBPT and SAT were presence and absence of agglutination which indicate positive and negative sample respectively.

Statistical analysis

The data generated from the study were subjected to simple percentages and Chi-Square (χ^2) tests using SPSS version 20 statistical software.

Results

An overall prevalence of 114 (11.4%) was recorded, out of which 51 (8.7%) were males and 63 (15.2%) females following RBPT and SAT. The seroprevalence based on sex above are statistically different (P<0.01) (Table 1). Seroprevalence of brucellosis in donkeys according to breeds showed that Duni has statistically higher prevalence (P<0.01) of 8 (18.2%) followed by Auroki 105 (11.5%) (Table 2). The prevalence of brucellosis according to age showed that donkeys that range from ≥7 years had the highest prevalence of 16 (19.8%), followed by donkeys ranging from 5-6 years (17.8%) (Table 3). Statistically higher prevalence (P<0.05) was recorded in the market area 34 (17%) with the least being Udubo east 16 (8%) following RBPT and SAT (Table 4).

Husbandry/ management factors affecting prevalence of brucellosis in donkeys indicates that herd size of 11-20 has the significantly higher seroprevalence (P<0.05) 32(19.2%), followed by herd size of 31-40 donkeys 6 (18.2%) then 41-50 herd size 3 (7.1%). Also, statistically higher prevalence (P<0.05) were recorded in extensive system of management 105 (11.5%), supplied drinking water from River/stream 90 (12.9%) and coming together of donkeys with other animal species 111(11.5%) than their counterparts (Table 5).

Table 1: Sex-Specific prevalence of brucellosis among donkeys based on rose bengal plate test (RBPT) and serum agglutination test (SAT)

Sex	Positive	Negative	Total	χ^2
Males	51 (8.7%)	534 (91.3%)	585	**
Females	63 (15.2%)	352 (84.8%)	415	

****= Significant at P<0.01**

Table 2: Breed-Specific prevalence of brucellosis among donkeys based on rose bengal Plate test (RBPT) and serum agglutination Test (SAT)

Factors Breed	Positive	Negative	Total	χ^2
Auroki	105 (11.5%)	809 (88.5%)	914	**
Duni	8 (18.2%)	36 (81.8%)	44	
Fari	0 (0%)	28 (100%)	28	
Idabori	1 (7.1%)	13 (92.9%)	14	

****= Significant at P<0.01**

Table 3: Age-Specific prevalence of brucellosis among donkeys based on rose bengal plate test (RBPT) and serum agglutination Test (SAT)

Year	Positive	Negative	Total	χ^2
≤2	12 (8.6%)	128 (91.4%)	140	**
3-4	54 (9.0%)	544 (91%)	598	
5-6	32 (17.8%)	148 (82.2%)	180	
≥7	16 (19.8%)	66 (80.5%)	82	

****= Significant at P<0.01**

Table 4: Location-Specific prevalence of brucellosis among donkeys based on rose bengal plate test (RBPT) and serum agglutination test (SAT)

Location	Positive (%)	Negative (%)	Total	χ^2
Gamawa East	27(13.5%)	173(86.5%)	200	*
Gamawa West	20(10%)	180(90%)	200	
UduboEast	16(8%)	184(92%)	200	
Udubo West	17(8.5%)	183(91.5%)	200	
Market	34(17%)	166(83%)	200	

***= Significant at P<0.05**

Discussion

The result in this study showed that female donkeys have higher prevalence. There was also significant association between sexes of donkeys and positive serological reaction in this study.

The higher prevalence of brucellosis obtained amongst female donkeys in this study was in consonance with the findings reported by (18) in horses in eastern Turkey. These findings are also in agreement with the works of (19, 20, 21, 22) on the seroprevalence of brucellosis by sex of camels who reported

higher prevalence in females than in males in northern Nigeria. (23, 24) also reported that the seroprevalence of brucellosis was two-folds higher in females compared to males in eastern Sudan. Furthermore, (25) reported that susceptibility increases after sexual maturity and pregnancy. The possible explanation for the higher prevalence in females could be due to the fact that female donkeys stay longer in the herd for the purposes of reproduction. However, (26) reported no difference in the prevalence of animal brucellosis between males and females. In addition, since brucellosis causes ochitis and epididymitis in males, they may be culled faster, except if abortion occurs in females, which may be difficult to detect clinically.

There are controversial reports regarding the prevalence of brucellosis in relation to the sex of animals, as some of the research workers reported significantly higher prevalence in males than females (27), while others reported that females have higher prevalence than males (28).

The prevalence according to breed showed that Duni had the highest prevalence of 18.2%, followed by Auroki 11.5%. The information available on the difference of brucellosis infection according to breed is scarce at the moment (30). The reason for the differences in prevalence found in this study requires further investigation. In swine, breeds such as Duroc and Jersey Red crosses may be less susceptible to experimental challenge with *B. suis*, suggesting some genetic resistance (30). It may be possible that the overwhelming number of one breed, Auroki (914/100) might have influenced the data masking the contribution of other less common breeds.

Table 5: Management/husbandry factors affecting prevalence of brucellosis in donkeys

Factors	Category	Total	Positive	χ^2
Herd size	1-10	579	60(10.4%)	*
	11-20	167	32(19.2%)	
	21-30	127	7(12.7%)	
	31-40	33	6(18.2%)	
	41-50	42	3(7.1%)	
	≥50	52	6(11.5%)	
Management system				
1. Intensive		0	0(0%)	*
2. Semi-intensive		89	9(10.1%)	
3. Extensive		911	105(11.5%)	
Source of drinking water				
1. River/Stream		697	90(12.9%)	*
2. Well		254	20(7.1%)	
3. Tap water		49	4(8.2%)	
Comingling with other species				
1. Yes		968	111(11.5%)	*
2. No		32	3(9.4%)	

***= Significant at P<0.05**

The distribution of prevalence according to age in this study reveals that donkeys within age group 7-8 years had the highest prevalence of 19.8%, followed by donkeys 5-6 years old 17.8%. Higher prevalence of brucellosis in donkeys within 7-8 years of age is in agreement with the findings of (31). This may be because older animals are in contact for a very long time with the infected animals and with the environment.

Based on location, donkeys from the market had the highest seroprevalence of 17%, followed by Gamawa East with 13.5%. Udubo East had the least prevalence of (8%). The higher prevalence obtained from the donkeys in the market could be attributed to the fact that donkey trading in Nigeria is usually of old, sick and exhausted animals that are brought to the market from far and wide. Other factors that may influence the differences in prevalence of brucellosis according to location in Nigeria include management system (32), the herding of different species together (33), use of common pasture and water sources (34).

This study recorded significantly higher prevalence of brucellosis within herds that have a herd size ranging from 11-20 with a prevalence of 19.2%, followed by herd size 31-40 with prevalence of 18.2%. The least prevalence (7.2%) according to herd size was recorded in herd size 41-50. Herds/household that practice extensive system of management had a higher prevalence of 11.5%, followed by semi-intensive with a prevalence of 10.1%. Prevalence of brucellosis according to source of drinking water showed that donkeys that were supplied drinking water from River/stream had the highest prevalence of 12.9%, followed by those that were supplied drinking water from the tap with a prevalence of 8.2%. This could be attributed to the fact that donkeys that drank from the river share the same drinking point with other species of animal. Larger herds might be expected to be associated with extensive management practices that are typically more difficult to control and examine for any suggestive symptom of disease, which increases the potential for exposure to infectious excretions (35).

In addition, the stressful conditions of animals subjected to extensive production may make them more susceptible to the infection. Since extensive management implies rearing a large number of animals in large areas and/or sharing communal pastures, the contamination of pastures and drinking water with placentas or abortion materials is a source of infection to other animals in the herds (36). This fact constitutes an important source of infection for humans and for the young animals. Introduction of animals from market fairs also presents a higher risk of infection (37). The majority of infections or re-infection in disease-free herds starts through buying infected animals of unknown status (35).This has a higher importance in those endemic areas or countries where there is an absence of control programmes. However, in countries with test-and-slaughter control programmes, the movement of cattle are subjected to a compulsory pre-movement test that consists in the serological brucellosis diagnostics before an animal leaves the farm. Moreover, animal movement restriction measures are applied in brucellosis positive herds to avoid spreading the disease.

The proximity to other infected herds or flocks has also been described as an infection risk, although, small ruminant contact with other flocks was reported to have no impact on *Brucella* seropositivity in Spain (38).

Furthermore, one major factor contributing to the spread of the disease is the free movement of animals (39). Other factors that may influence the prevalence of brucellosis in Nigeria include management system (32), the herding of different species

together (33), use of common pasture and water sources (34), age (39, 41), breed (42), sex, lactation status (33) and season (34). Donkeys were allowed to mingle among other species of animals in the study area, and donkeys are allowed to graze freely with little or no feed supplement. There is scarcity of information regarding husbandry and management of donkeys (42).

Conclusions and Applications

It can be concluded from the findings of this study that:

1. There is a high prevalence of brucellosis among donkeys in the study area.
2. Sex, breed, age, location and management practices of donkey influence the prevalence of brucellosis in the study area.
3. Government coordinated public awareness on brucellosis in donkeys with emphasis on its economic and public health implications are hereby recommended.
4. There is need for improved sanitary measures with proper handling and disposal of after birth and aborted foetuses by burning and deep burying in the ground.
5. Identification of donkeys with brucellosis makes it imperative that strategic planning for the control of brucellosis in Nigeria should include donkeys.
6. Test and slaughter policy with compensation to the farmers is recommended for those that tested positive. This will reduce the number of positive cases and therefore reduce the spread of the infection to other healthy animals and humans.

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