Sjournals
Scientific Journals
Scientific Journal of Microbiology (2012) 1(4) 91-96

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Prevalence of *brucella abortus* antibodies in bovine serum from gusau modern abattoir, Zamfara state, Nigeria

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ARTICLE INFO

Article history:
Received 17 September 2012
Accepted 25 September 2012
Available online 30 September 2012

Keywords:
Abattoir
Brucella abortus
Antibodies
Serum
Elisa
White Fulani
Arzuwaq
Sokoto Gudali
Cross Breed
Gusau
Zamfara
Nigeria

ABSTRACT

A study for bovine brucellosis was conducted using serology to determine the status of the disease in slaughtered cattle. Three hundred and twenty (320) sera were collected from Gusau Modern Abattoir, Zamfara State. The sera were tested using Enzyme Linked Immunosorbent Assay obtained from Veterinary Laboratory Agencies, Weybridge, UK. An overall prevalence of 20% (64 positive) was obtained with sex prevalence for males and females being 10.62% (34 positive) and 9.37% (30 positive) respectively out of 180 males and 140 females tested without significant association ($P < 0.05$). On age distribution, higher prevalence of 11.87% was recorded in age group > 24 months while; lower prevalence of 3.13% was recorded in age group < 12 months. There was no significant association statistically between age and occurrence of antibodies. White Fulani breed had the highest prevalence of 8.75%. There was significant association statistically ($p < 0.05$) between breed and infection.

1. Introduction

Brucellosis is a bacterial disease caused by *Brucella* species in both animals and man. They are obligate intracellular parasites that are Gram negative, non-motile, non-spore forming rods or coccobacilli that are small in size and become localized in the reticuloendothelial systems and reproductive tract, causing various illnesses such as abortion, sterility, orchitis, epididymitis and hygroma in animals, and undulant fever, malaise and sterility in man (Glenn songer and Post, 2005). The aetiologic agents of the disease have host preference with *Brucella melitensis, B. abortus, B. suis, B. canis* and *B. neotomae* infecting goats, cattle, swine, dogs and wood rats respectively (Hirsh and Yuan, 1999). Cross infection among animals in close contact have been reported (Musa *et al*., 2008). Brucellosis is a disease causing great economic losses not only to the farmers but to the country's economy, due to reproductive failure and low survival of weak neonates born to infected dams (Ocholi *et al*., 2005). The impact of brucellosis in the north where the highest population of the country's livestock is located cannot be over emphasized due to high economic losses (Esuruoso, 1979), this is because apparently healthy animals harboring the organisms could be a source of infection to hitherto brucella free herds especially during animal replacement, as little or no attention is given to testing of animals for diseases before purchase (Shehu *et al*., 1999).

Brucellosis has been reported in cattle, sheep, goats, camels, chicken and humans in various parts of Nigeria; thus indicating the magnitude of the disease in the country (Junaidu *et al*., 2008; Salihu *et* al., 2011). Varying prevalence rates in different parts of the country and even within the same geographical zones operating different husbandry techniques have been reported (Esuruosu, 1974). The disease has a worldwide distribution, affecting humans and animals both in the developed and developing countries (Mukhtar and Kokab, 2008). Brucellosis is more of a problem in the developing countries due to lack of: effective public health measures, domestic animal health programs and appropriate diagnostic facilities (Mukhtar and Kokab, 2008). The situation is compounded by the presence of other diseases having clinical signs resembling that of the disease leading to incorrect diagnoses and under-reporting of the disease (Thakur *et al*., 2002). Bovine brucellosis is said to be endemic in Nigeria having been reported in the country as far back as 1920s in several sporadic outbreaks. The first cases were reported by Anon (1927) and Earnshaw and O'Brien (1928) in cattle in separate sporadic outbreaks in government owned farms. More of the reported cases were from established farms than from the nomadic cattle rearers who control about 90% of the animal population (Cadmus *et al*., 2006). Evidence of the disease has been documented in various animals' species including man such as cattle (Bale and Kumi Diaka, 1981), horses, cattle, sheep and goats (Ocholi *et al.,* 2004a, 2004b), sheep (Ocholi *et al*., 2005), sheep and goats (Bertu *et al.*, 2010), cattle, sheep, goats, pigs and humans (Cadmus *et al*., 2006), cattle, sheep, goats, chicken and humans (Junaidu *et al*., 2006, 2008, 2010; Salihu *et al*., 2011). Despite these extensive researches undertaken, little information is available on the status of the disease in Zamfara state. The objectives of this study were to investigate the status of cattle slaughtered in Gusau modern abattoir on brucellosis and determine some epidemiological variables such as sex, age and breed as they relate to the disease.

2. Materials and methods

2.1. Study area

Zamfara is located at Lattitude 11º 10' N and Longitude 6º 15' E, covering an area of 39,762 km^2 with an estimated population of 3, 582, 912with Gusau as its capital (UNEP/OCHA,2010). The climate is semi-arid with temperature above 28.5°C, annual rainfall of less than 1000 mm and relative humidity below 70% (Odjugo, 2010). It shares borders with Kebbi, Kaduna, Sokoto, Niger and Katsina states. It also shares an international boundary with Niger Republic to the north (UNEP/OCHA, 2010). Majority of the people are engaged in farming and livestock rearing. Gusau Modern abattoir is located along Gusau - Sokoto road in the outskirt of Gusau town, opposite Nigeria National Petroleum Corporation (NNPC) Depot, Gusau.

2.2. Sampling procedure sample sise

Systematic random sampling procedure as described by Thrusfield (1997) was used in selecting animals for sample collection and 320 blood samples were collected as determined using the formula described by Daniel (1999).

2.3. Blood sample collection

Five milliliter (5 ml) of blood samples were collected from 320 selected cattle in a sterile bijou bottles and kept in an icepack in slanting position to allow for blood clotting to harvest the serum. The samples were immediately transported to the laboratory.

2.4. Sample processing

In the laboratory, the serum samples were centrifuged at 5000 rpm for 10 minutes to further obtain a clear serum. The serum samples were then decanted into 5 ml, sterile blood sample bottles and stored in a deep freezer at -20˚ C until analyzed.

2.4.1 Enzyme linked immunosorbent assay (ELISA) test

An indirect Enzyme Linked Immunosorbent Assay kit BRUCELISA 160 obtained from Veterinary Laboratories Agency, Weybridge, UK was used to test the serum samples. The test was conducted according to the manufacturer's instructions. Initially the diluting buffer, wash solution, stopping solution, conjugate and controls were reconstituted as instructed by the manufacturer. Test serum was added per each well of the microtitre plate which has 96 wells. Wells in columns 11 and 12 were used as controls.

A primary dilution of 1/40 of all test and control sera was done by adding 25 µl of serum to 1 ml of diluting buffer. The plates were prepared by adding 80 µl of diluting buffer to all wells. Twenty microlitre (20 µl) of each of the primary diluted samples was added to all prepared wells. A final dilution of 1/200 was obtained. Twenty microlitre (20 µl) of the primary diluted positive control was added to each of the wells in column 11. Another 20 µl of the primary diluted negative control was added to all the wells in column 12 except H12 which was left to blank the plates. The plates were covered with lids and incubated at 37˚ C for 1 hour. After 1 hour of incubation, the plates contents were shaken out and the plates were rinsed 5 times with washing solution and then dried thoroughly by tapping gently with absorbent paper towel. One hundred microlitre (100 µl) of the conjugate solution was added to each well and the plates were covered with lids and incubated at 37˚ C for another 1 hour. After the incubation time, the plate's contents were shaken out and rinsed 5 times with washing solution and then dried again with absorbent paper. Immediately, the substrate solution was prepared by adding 300 µl of ABTS chromogen to 12 ml substrate buffer and 60 µl of substrate (Hydrogen Peroxide). They were mixed well and 100 µl was added to each well. The plates were left at room temperature for 10 to 15 minutes before the reaction was slowed by adding 100 µl of stopping solution to all wells. Condensation was removed from the bottom of the plates with an adsorbent paper towel and the plates were visually inspected for color change to determine positive and negative sera. The color of the test wells were compared with the control wells and the results recorded.

2.5. Statistical analysis

Data obtained were subjected to statistical analysis using Chi-square for test of significant association using the formula as described by Swinscow (1997).

3. Results and discussion

A total of 320 cattle were tested out of which 180 were males (56.25%), while 140 were females (43.75%). Of this number, 64 sera (20%) were positive. On sex distribution, 34 were males and 30 were females with prevalence of 10.625% and 9.375% respectively. There was no significant association (χ^2= 0.31, p> 0.05) between *Brucella abortus* infection and sex (Table 1).

Age Distribution: Out of the 320 cattle tested, 40 (12.5%) were within the age band of less than 12 months and 10 were positive with prevalence of 3.12%, 48 (15%) were in the age band of 13 – 24 months with 16 being positive giving a prevalence of 5.00% and 232 (72.5%) were in the age band greater than 24 months with 38 positive giving a prevalence of 11.875%. There was no significant association (χ^2=2.221, P > 0.05) between *Brucella abortus* infection and sex (Table 2).

Breed Distribution: The four breeds encountered during the study were 138 White Fulani (43.12%) with 28 positive, 52 Arzuwaq (16.25%) with 10 positive, 74 Sokoto Gudali (23.12%) with 10 positive and 56 Cross Breed (17.5%) with 16 positive.The prevalence among breeds was 8.75%, 3.12%, 3.12% and 5.00% for White Fulani,

Arzuwaq, Sokoto Gudali and Cross Breed respectively. There was no significant association (χ^2= 4.571, P > 0.05) between *Brucella abortus* infection and breed (Table 3).

Table 1
Number of slaughtered cattle positive for *Brucella abortus* using ELISA in Gusau Modern abattoir, Zamfara State based on sex.

Sex	No. of Animals N	Positive N (%)	Negative N (%)
Males	180	34 (10.62)	146 (45.62)
Females	140	30 (9.37)	110 (34.37)
Total	320	64 (20.00)	256 (80.00)

χ^2= 0.31, p > 0.05

Table 2
Number of slaughtered cattle positive for *Brucella abortus* using ELISA in Gusau Modern abattoir, Zamfara State based on age.

Age (Months)	No. of Animals N	Positive N (%)	Negative N (%)
<12	40	10 (3.13)	30 (9.37)
13 – 24	48	16 (5.00)	32 (10.00)
> 24	232	38 (11.87)	194 (60.63)
Total	320	64 (20.00)	256 (80.00)

χ^2=2.221, p > 0.05

Table 3
Number of slaughtered cattle positive for *Brucella abortus* using ELISA in Gusau Modern abattoir, Zamfara State based breed.

Breed	No. of Ainmals N	Positive N (%)	Negative N (%)
White Fulani	138	28 (8.75)	110 (34.37)
Arzuwaq	52	10 (3.12)	42 (13.12)
Sokoto Gudali	74	10 (3.12)	64 (20.00)
Cross Breed	56	16 (5.00)	40 (12.50)
Total	320	64 (20.00)	256 (80.00)

χ^2= 4.571, p > 0.05

The high seroprevalence in the sera of slaughtered cattle tested positive by ELISA 20.00% observed in this study agrees with the results obtained by Junaidu (2010), who reported a prevalence of 22.38% in slaughtered cattle in Sokoto. This high prevalence may be due to the high population of livestock in the study area similar to the high livestock population as obtained in the neighbouring Sokoto state (Bourn *et al*., 1992; FAO, 2006). When compared with the prevalence rates of 5.82% reported by Cadmus *et al*. (2006) in Ibadan, 10.80% and 10.00% reported by Shehu *et al*. (1999), and Ocholi *et al*. (2005) both in Bauchi, the prevalence obtained in this study is higher. The high prevalence rate recorded in this study may be linked with the absence of annual vaccination programme, improper hygienic practices in disposing aborted foetuses, placenta and vaginal discharges which contain virulent *Brucella abortus* , thus contaminating the pasture and water, serving as reservoirs of infection to other cattle as observed by Corbel (1985) and Salihu *et al*. (2011). Similarly, because the cattle brought for slaughter in the study area were mainly sourced from markets, increase prevalence reported in this study may be associated with indiscriminate gathering of sick and healthy animals from different farms, states and neighbouring Niger Republic for sales as trade cattle, as this may facilitate the transmission of diseases from sick to healthy animals, particularly during transportation as observed by Cadmus *et al*. (2006).

The higher prevalence recorded in the males (10.62%) than in the females (9.37%) had no significant association between sex and *Brucella* infection (p> 0.05) and is in contrast with the findings of Salihu *et al*. (2011),

where they recorded higher prevalence in the females than in the males. This may be due to the fact that most female animals tested were younger and reports have shown that younger animals tend to be more resistant to brucellosis (Hirsh and Yuan, 1999; Chukwu, 1987). The lower prevalence rates recorded in the age groups less than 12 months (3.13%) and 13-24 months (5.00%) compared to those greater than 24 months (11.87%) respectively showed no significant association statistically between *Brucella* infection with age ($p>0.05$) and is in disagreement with the findings of Salihu *et al*. (2011), where they recorded higher prevalence in older cattle. This may be due to their high susceptibility to infection as a result of prolonged exposure (Salihu *et al*., 2011). Similarly, it was reported that younger animals tend to be more resistant to infection and frequently eliminate same, while sexually matured animals are much more susceptible to infection regardless of gender (Hirsh and Yuan, 1999; Chukwu, 1987).

This study recorded higher prevalence in the White Fulani breed (8.75%), followed by Cross breed (5.00%), Sokoto Gudali (3.12%) and Arzuwaq (3.12) but did not show significant association statistically ($p>0.05$). This appears to be in disagreement with the findings of other researchers (Junaidu *et al*., 2008; Salihu *et al*., 2011) in the neighboring Sokoto state, where Sokoto Gudali was found to be the breed with the highest prevalence, but may not necessarily be so, since both White Fulani and Sokoto Gudali are predominantly kept by the nomadic Fulanis in the study area for milk production, and brucellosis was reported to be on the increase in nomadic Fulani herds in Nigeria (Rikin, 1988).

4. Conclusion

The identification of brucella antibodies in sera of slaughtered cattle in the abattoir is significantly established the presence of infection in the slaughtered cattle and it constitutes a public health concerns due to possible exposure and infection to the butchers, as the role of carcasses and their edible offal as means of infection was reported (Sadler, 1960). Similarly, because the cattle brought for slaughter in the study area were mainly sourced from markets, increase prevalence reported in this study may be associated with indiscriminate gathering of sick and healthy animals from different farms, states and neighboring Niger Republic for sales as trade cattle, as this may facilitate the transmission of diseases from sick to healthy animals, particularly during transportation as observed by Cadmus *et al*. (2006).

The butchers and Fulani herdsmen and their families are at high risk of exposure and infection due to their occupation, food habits and closeness to the cattle (Salihu *et al*., 2011). Based on these there is the need for improved hygienic measures such as proper disposal of aborted fetal materials and protection of livestock farmers and veterinarians as well as periodic screening to determine their health status as observed by Salihu *et al*. (2011). To achieve this, public enlightenment on the dangers of brucellosis should be embarked upon by the appropriate stakeholders to help in reducing risk of infection especially among high risk group, government should embark upon mass vaccination of cattle of all ages except pregnant animals to help develop herd immunity against brucellosis in the state and access to serological tools and funds should be provided for veterinary personnel in the abattoir for quick serological diagnosis of brucellosis and subsequent elimination of infected animals with the co-operation of enabling authorities.

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