



PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN THREE REGIONS OF GHANA

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Summary

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Information on the prevalence of important reproductive zoonoses in West African countries other than Nigeria had been scanty in literature. This study estimates the prevalence of brucellosis in sheep and goats from Northern, Ashanti and Greater Accra regions of Ghana. Tissues/swabs (319) and serum (370) samples were collected from sheep and goats comprising male and female West African Dwarf (WAD) and Sahelian breeds between 1 to 4 years of age in the regions. These were screened for brucellosis using Modified Ziehl Neelsen (MZN) staining method and the Rose Bengal Plate Test (RBPT). A seroprevalence of 13.3% was recorded while 17.0% were positive with modified ZN staining. Goats (10%) and female animals (7.0%) had slightly higher seroprevalence. Adult animals of above two years had prevalence of 92.0% while WAD breed had the highest prevalence of 63.0% with 2.7% in female WAD. More seropositive animals were found from Ashanti region than other regions. Due to the zoonotic and economic implications of the disease, there is the need to embark on the strict control strategies including vaccination of small ruminants against brucellosis.

Key words: brucellosis, Ghana, MZN staining method, seroprevalence, small ruminants

INTRODUCTION

Ghana hosts a large number of small ruminants that are raised under extensive pastoral production system and in adjunct to crop production (Karbo & Agyare, 2000). Despite the country's vast resources of forage, its livestock resource

base is modest with about 1.3 million cattle, 2.5 million sheep and 2.7 million goats (ADF, 2001). The total estimated livestock population of Ghana is about 58.4 million of which Northern Ghana accounts for over 50% of agricultural

production, while Ashanti and Greater Accra are of the receiving end in the agricultural production chain due to the large human population (SRID, 2010).

Ghana seems not to optimally utilise this resource as the country still relies on major import of small ruminants (Karbo & Agyare, 2000). Despite this obvious limitation, disease is a major factor that limits the economic return from small ruminant production. A disease that hampers the productivity of small ruminants is brucellosis (Megid *et al.*, 2010).

Brucellosis is recognised as one of the most important zoonotic diseases across the world as it poses a major threat to both human health and animal populations (Cutler & Cutler, 2006; Mensal *et al.*, 2011). The World Health Organization considers it as a neglected zoonosis, because adequate control programmes were not in place in numerous countries despite its huge impact on human and animal health, also on the economy (Holt *et al.*, 2011). Brucellosis poses a barrier to the trade of animals and their products, and the associated reproductive wastage/infertility is enormous (Godfroid *et al.*, 2011).

Small ruminant brucellosis is mostly caused by *Brucella melitensis* (Prahad *et al.*, 1997; Omer *et al.*, 2002). *Brucella ovis* is also important, causing orchitis and epididymitis in rams but it is not a cause of natural infection in goats (Smith & Sherman, 1996). Persistent infection is a common feature of the disease with frequent shedding of the bacterium in body secretions (Tanko *et al.*, 2013). Brucellosis has been reported in small ruminants from different parts of the world in Somalia (Falade & Hussein, 1997), Eritrea (Omer *et al.*, 2000) Ethiopia (Teshale *et al.*, 2006), Sudan (El-Ansary *et al.*, 2001) and Nigeria (Shehu *et al.*, 1999). In Ghana, Aning *et al.* (2002) reported a

high prevalence of *B. abortus* antibodies in raw cow milk taken from retailers and wholesalers in Accra and Kumasi. Also, the disease in cattle was reported from the coastal and northern savannah zones (Turkson & Boadu, 1992) as well as the middle forest belt by Kubuafor *et al.* (2000). The detection of *B. abortus* as well as several other pathogens in raw milk from Ghana in the past has not raised the needed concern as expected (Ayebo & Assoku, 1976; Mensal *et al.*, 2011) and in recent years, the marketing of fresh dairy from cattle and goat in Ghana, especially in the urban and peri-urban areas has increased considerably in response to a growing demand for such products. The dearth of information on this disease and its prevalence in small ruminants require the need to study its occurrence, for generation of baseline data and policy formulation on zoonosis control in small ruminants and other livestock.

MATERIALS AND METHODS

Study area

The study was carried out in three regional abattoirs and slaughter house; Accra, Kumasi and Tamale between October and November 2013. Tamale municipal abattoir is located in Shishegu, a suburb of Tamale Metropolis between latitudes 9°15' – 9°30'N and longitudes 0°45' and 10°W. Kumasi abattoir is located at 6°39'36.6"N Latitude and 1°36'15.4"W Longitude, in the Kumasi city of Ghana, and the Accra slaughter house is in Accra metropolitan area.

Animals

A total of 370 sheep and goats were sampled randomly across the three regions. These comprised the West African dwarf

(WAD) and Sahelian breeds of both species common in the regions. They were categorised as young adults (less than 2 years of age) and adults (above 2 years of age). Goats consisted of 268 adults and 18 young adults and sheep – of 72 adults and 12 young adults.

Serum collection

A cross-sectional study was conducted to determine the occurrence of brucellosis in small ruminants. Serological tests and questionnaire survey were used as a tool to determine the prevalence and assess the associated risk factors. Blood samples were collected from the sheep and goats in a simplified random approach before slaughter. Approximately 7–10 mL of blood was collected from jugular vein using plain vacutainer tubes and needles. Individual tubes were identified using numbers and alphabets to indicate their location and source. The tubes were left tilted overnight at room temperature to allow clotting. The sera were separated from the clot (unretract blood centrifuged) by siphoning into sterile test tubes. The 370 serum samples from 286 goats and 84 sheep were transported in icebox to the Veterinary laboratory in Accra and stored at –20 °C until Rose Bengal Plate Test (RBPT) was carried out using standard techniques.

Tissue /swab collection

Tissues from placenta (6), foetal fluid (37), vaginal (100), preputial (38) and nasal (138) swabs were screened using Modified Ziehl Neelsen (MZN) staining technique as described by Balows *et al.* (1991) & Miller (1991). The distribution of all animals examined is presented in Table 1.

Modified Ziehl Neelsen acid fast stain

Tissues and swabs were smeared lightly on glass slides and allowed to air dry for a few minutes. The smeared slides were fixed in 70% methanol for 10 minutes. The slides were placed on staining rack and flooded with carbol fuchsin and allowed to stain for 3 minutes before thorough rinsing with water. Slides were de-colourised with 5% sulphuric acid for 30 seconds, rinsed with water, counterstained with methylene blue for 20 seconds and finally rinsed with water and air dried. Stained smears were examined under Olympus microscope using oil immersion for detailed morphology. Samples with stained characteristic red-pinkish coccobacilli were recorded positive (Balowa *et al.*, 1991).

Rose Bengal plate test

It was carried out using standard Rose Bengal Plate Test antigen obtained from

Table 1. Number and percentage of sheep and goats examined

| | Goats | | Sheep | | Total |
|--------|-----------|----------------|-----------|----------------|------------|
| | WAD breed | Sahelian breed | WAD breed | Sahelian breed | |
| Male | 52 (14%) | 74 (21%) | 24 (6%) | – | 150 (41%) |
| Female | 72 (20%) | 88 (23%) | 58 (15%) | 2 (1%) | 220 (59%) |
| Total | 124 (34%) | 162 (44%) | 82 (21%) | 2 (1%) | 370 (100%) |
| | 286 (78%) | | 84 (22%) | | |

Central Veterinary Laboratory, Weybridge U.K.PA 0060 batch 281, according to the method of Alton *et al.* (1975). Equal volumes (0.03 mL) of antigen and test serum were mixed thoroughly on the glass plate of the test box using a tooth pick and the box was hand rocked for 4 min. Samples that showed signs of agglutination were recorded as positive while those with no sign of agglutination were recorded negative.

RESULTS

The number and percentages of small ruminants examined are presented in Table 1. The study cohort comprised 78% goats and 22% sheep.

Smears from the foetal fluid and impressions from the placenta revealed red stained cocco-bacilli in pairs and a few chains. Smears from the upper respiratory tracts were negative; 17% of smears were positive for *Brucella* sp (reddish stained

coccobacilli) – Table 2.

Serologically, there was a prevalence of 10.3% for goats and 3% for sheep. The number and percentage distribution based on sex and species of sheep and goats are presented in Table 3.

Of these 49 serologically positive animals; 45 were adult above two-year-old (92%) and 4 young adults (8%). Thirty-one were from the WAD breed (63%) and 18 – from the Sahelian breed (31%). More serologically positive animals were recorded from Kumasi, followed by those in Accra and Tamale (Table 4).

Gross examination of carcasses revealed that few of the female animals had bloody foul smelling discharges from their vulva and the uterus containing abundant odourless, dirty yellow, slightly viscid and slimy exudates. In a few gravid animals (a goat and three sheep), the uteri contained gray-yellow, pulpy floccules of detritus in between the endometrium and chorion in the intercotyledonary area. Their foetal

Table 2. Number and percentage of *Brucella* positive smears from sheep and goat using the modified Ziehl Neelsen staining test.

| Tissue/swab | Goats | Sheep | Number of smears | Positive smears |
|--------------|-------|-------|------------------|-----------------|
| Foetal fluid | 29 | 8 | 37 | 18 (5.6%) |
| Placenta | 4 | 2 | 6 | 3 (0.9%) |
| Vaginal | 73 | 27 | 100 | 20 (6.3%) |
| Preputial | 31 | 7 | 38 | 12 (3.8%) |
| Nasal | 107 | 31 | 138 | 0 |
| Total | 244 | 75 | 319 | 53 (17%) |

Table 3. Number & percentage of animals positive in the Rose Bengal Plate Test.

| | Goats | | Sheep | | Total |
|--------|------------|----------------|-----------|----------------|------------|
| | WAD breed | Sahelian breed | WAD breed | Sahelian breed | |
| Male | 11 (3%) | 11 (3%) | 1 (0.3%) | – | 23 (6.3%) |
| Female | 9 (2.4%) | 7 (1.9%) | 10 (2.7%) | – | 26 (7%) |
| Total | 20 (5.4%) | 18 (4.9%) | 11 (3%) | – | 49 (13.3%) |
| | 38 (10.3%) | | 11 (3%) | | |

Table 4. Number & percentage positive of animals from different locations (Rose Bengal Plate Test)

| Location | Screened small ruminants | Positive goats (%) | Positive sheep (%) | Total positive (%) |
|----------|--------------------------|--------------------|--------------------|--------------------|
| Accra | 120 | 7 (1.9%) | 4 (1.1%) | 11 (3.0%) |
| Kumasi | 196 | 27 (7.3%) | 6 (1.6%) | 33 (8.9%) |
| Tamale | 54 | 3 (0.8%) | 2 (0.6%) | 5 (1.4%) |
| Total | 370 | 37(10%) | 12 (3.3%) | 49 (13.3%) |

membranes and the umbilical cord were thickened and saturated with clear oedema fluid. The placental lesions were not uniform; some cotyledons appeared more or less normal and others extensively necrotic. A ram was found with moderate unilaterally inflamed testis (orchitis). Additional lesions included enlarged spleen and lymph nodes with few animals having severely pneumonic lungs and congested liver.

DISCUSSION

This appears to be the first extensive study on the prevalence of brucellosis in small ruminants from major regions of Ghana. The study showed higher prevalence of *Brucella* antibodies in goats than in sheep. Similar prevalence was observed in female and adult animals (above 2 years) especially of WAD breed. Occurrence in these animals further supports susceptibility of reproductive animals and the risk goat pose in the spread of brucellosis compared to sheep (Godfroid *et al.*, 2011).

The current study revealed higher prevalence of *Brucella* antibodies (13.3%) in the absence of *Brucella* vaccination. This is quite higher than prevalence rates of 1.7% in sheep and 1.5% in goats in Sudan (Abdala, 1966), 3.8% in goats and 1.4% in sheep in Eritrea (Omer *et al.*, 2000) and 4% in goats and 1% in sheep in eastern Sudan (El-Ansary *et al.*, 2001). Similar to 6.01% in sheep and goats in

Kenya (Waghela, 1976), 2.8 and 5.29% in goats and 7.2% in sheep in Somalia (Falade & Hussein, 1997), 13.2% in goats and 5.6% in sheep in Ethiopia (Teshale *et al.*, 2006) and 6.6 % in sheep and 4.75% in goats in Nigeria (Shehu *et al.*, 1999). This is an indication of the wide spread nature of *Brucella* infection in Africa and a need for workable strategies for the control of the disease.

The observed difference in the prevalence of 17% from tissue/swabs using MZN and 13.3% using RBPT may have underscored the relative specificity of the serological test and the sensitivity of MZN in detecting *Brucella* antigen in tissues and secretions.

The prevalence of *Brucella* in goats from all the regions may be attributed to the uncontrolled influx from neighbouring countries, the grazing and housing of cattle with sheep and goats which increases the chance of transmission of the disease (Seleen *et al.*, 2010). The detection of higher antibody titres in does than in bucks further suggests that female animals are generally more susceptible to *Brucella* infection than the males (Keppie *et al.*, 1965).

With the economic loss resulting from brucellosis placed at \$223.2 million in Nigeria (Esuruoso, 1977), the prevalence rate of brucellosis in goats and sheep as observed in this study may lead to high economic loss and serious public health implications in Ghana. This therefore calls

for the introduction of stringent control measures including mass vaccination for small ruminants. Large-scale sero monitoring of ruminants and humans especially personnel workers at risk should be done to assess the level of infection in both animals and humans in Ghana. Further studies are focused on the isolation and molecular characterisation of the agent in these species.

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