Seroprevalence of antibodies against bacterial pathogens in sheep from Equatorial Guinea

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Summary

Equatorial Guinea (EG) is a country in central Africa with typical tropical weather. Sheep are an important source of food in EG, but the absence of information regarding infectious diseases that affect the native flocks of small ruminants is a concern. The country is currently implementing several new practices in the sheep industry associated with the importation of sheep from other countries. This study aimed to evaluate the seroprevalence of bacterial infections that are important to the sheep industry in EG sheep flocks. The detection of specific antibodies for the four agents studied was performed using enzyme-linked immunosorbent assay (ELISA) in 1,002 serum samples from EG sheep. The results showed a true prevalence of 13.37% for

antibodies against *Corynebacterium pseudotuberculosis*, 0.59% for *Brucella ovis*, 19.89% for *Chlamydophila abortus* and 0.79% for *Mycoplasma agalactiae* in animals from production flocks. Among a group of 35 samples from isolated native animals, 47.56% were seropositive for antibodies against *C. pseudotuberculosis*, 42.84% for *B. ovis*, 54.28% for *C. abortus* and 11.35% for *M. agalactiae*. These results comprise the first report of the prevalence of infectious diseases in sheep in EG. They highlight the importance of adopting adequate measures to prevent infection by bacteria endemic to EG native flocks during the development of the sheep industry in the country.

Keywords

Caseous lymphadenitis – Contagious agalactia – Enzootic abortion – Equatorial Guinea – Ovine brucellosis.

Introduction

The Republic of Equatorial Guinea (EG) is located in West Africa (3°21′0″N latitude and 8°40′0″E longitude). It has a typical tropical climate, hot and rainy (26°C and 2,500 mm rain per year, on average) with high air humidity (80–90%). According to the Food and Agriculture Organization of the United Nations (FAO) (1), the estimated sheep population of EG comprises 5,000 animals, which are usually maintained in an extensive system. However, there are no published scientific studies regarding livestock diseases in the country.

Caseous lymphadenitis (CLA), caused by *Corynebacterium pseudotuberculosis*, is highly prevalent in many countries worldwide and causes decreases in weight, lactation and commercial value of the fleece and, in some cases, infertility (2). In sheep, *Brucella ovis* infection leads to reduced fertility, management problems in the breeding season, abortion, epididymitis, orchitis and high rates of mortality among newborn lambs (3). Enzootic abortion of goats and sheep (EAGS), caused by the bacterium *Chlamydophila abortus*, is a zoonosis and a major cause of abortion, stillbirth, retained placenta,

metritis, persistent cough, polyarthritis and keratoconjunctivitis (4), as well as a factor for consideration during animal transit (5). *Mycoplasma agalactiae* is the aetiological agent of contagious agalactia of goats and sheep (CAGS), a disease that causes high levels of morbidity and mortality; it is associated with mastitis, agalactia, arthritis, keratitis, reduced milk production and transient fever (6).

The economic losses caused by these four diseases are considerable (7, 8). Given the lack of information on infectious diseases in EG, and the fact that the country is currently investing in the sheep industry, the aim of this study was to evaluate the prevalence of these four infections in EG sheep flocks.

Materials and methods

Based on FAO estimates (1), the minimum sample size '*n*' was calculated as proposed in (9), with a maximum sampling error of 5% and a 95% confidence level. With the aim of understanding the spread of the four diseases in the growing EG flock, samples were collected from several cities belonging to three of the four continental provinces, as shown in Fig. 1. There are no data on sheep breeding in the insular provinces of EG.

Insert Fig. 1 near here

Serum samples were collected from 1,002 sheep between 2013 and 2014, with the producers' consent. These serum samples were divided into two major groups: 967 sheep from properties that are investing in breeding enhancements, where sheep are raised in a semi-intensive management system, composed the first group; the animals that belong to these properties and were included in this study are herein referred to as 'production animals'. Thirty-five native geographically isolated sheep composed the other group; there are no reports of contact of these animals with other production animals, except wildlife. As these animals constitute a distinct group that may represent the infectivity profile of sheep in EG before the importation of other animals, these sheep are herein referred to as 'native animals'. Blood samples were collected in vacuum tubes, centrifuged at

1,500 ×*g* for 10 min to obtain serum, distributed in 1.5 ml aliquots and stored in a freezer at –20°C.

Serum samples were tested using an indirect ELISA with 99% specificity and 89% sensitivity, as described in (10), with the objective of identifying the presence of immunoglobulin (Ig)G antibodies against *C. pseudotuberculosis*. Briefly, polystyrene microplates (Corning Life Sciences, Lowell, United States of America [USA]) were sensitised with *C. pseudotuberculosis* secreted/excreted antigen diluted 1:100 in carbonate–bicarbonate buffer (pH 9.6) and incubated at 4°C overnight. The plates were then washed twice with PBS 0.05% Tween 20 (PBST), blocked with 5% skimmed milk in PBST, and incubated at 37°C for 2 h. After two additional washes with PBST, 100 µl of serum diluted 1:100 in PBST with 1% skimmed milk was added and the plates were incubated at 37°C for 1 h. Each serum sample was tested in duplicate. The plates were washed four times with PBST and a rabbit anti-ovine IgG antibody conjugated to horseradish peroxidase (Serotec, Raleigh, USA), diluted 1:20,000, was added and incubated at 37°C for 45 min. After incubation, the plates were washed and the reaction was developed with 100 µl/well of a solution containing hydrogen peroxide and tetramethylbenzidine (Moss Inc., Pasadena, USA) for 15 min, and stopped with 4 N sulphuric acid. The results were read using an ELISA plate reader (Bio-Rad Laboratories, Hercules, USA) at 450 nm.

To identify the presence in sera of specific IgG antibodies against *B. ovis*, *C. abortus* and *M. agalactiae*, commercial indirect ELISA kits were used (Idexx Laboratories Inc., Westbrook, USA), all of which had 99.9% sensitivity and specificity, as reported by the manufacturer. The assays were performed following the manufacturer's protocol. All sera that tested positive for *B. ovis* antibodies had their status confirmed by agar gel immunodiffusion (AGID) (Tecpar-PR, Curitiba, Brazil), following the protocol described by the manufacturer, with 96.4% sensitivity and 100% specificity (11). The true prevalence rates, adjusted for the sensitivity and specificity of each assay, were obtained by following the methodology described in (12).

Results and discussion

The results are presented in Fig. 2. An overall true seroprevalence of 19.31%, 19.83%, 0.65% and 0.83% for *C. pseudotuberculosis*, *C. abortus*, *B. ovis* and *M. agalactiae*, respectively, was found in sheep in EG. In production animals (n = 967), true prevalence values of 13.37%, 19.89%, 0.59% and 0.79% were estimated, while the isolated native animals (n = 35) showed true prevalence values of 47.56%, 54.28%, 42.84% and 11.35% for specific antibodies against *C. pseudotuberculosis*, *C. abortus*, *B. ovis* and *M. agalactiae*, respectively.

Insert Fig. 2 near here

The traditional extensive management system, which is commonly used in underdeveloped and developing countries, contributes not only to low productivity in the flocks, but also to the maintenance of many diseases (13). Moreover, the subclinical characteristics of the diseases studied here, and the lack of scientific information and infection reports in many of these countries are factors that must be taken into consideration when establishing government programmes for development and extension of the sheep industry.

The high seroprevalence of *C. pseudotuberculosis* and *C. abortus* in the native animals indicates the endemic presence of these bacteria in EG. This may be explained by the extensive management system, the absence of proper hygiene and the presence of risk factors associated with these infections in EG (14). There was also a significant prevalence of these infections in the animals in production flocks. According to (4), EAGS has a significant prevalence in all regions where goats and sheep are bred, which was confirmed in this work.

The results of this study showed that a high proportion of the isolated native animals were seropositive for *B. ovis*, but this was not replicated in the production animals. The low prevalence of the disease in production animals can be explained by the fact that the countries of origin of these animals (many were imported to EG as

part of a sheep industry implementation programme) perform rigorous brucellosis control measures.

The low seroprevalence of *M. agalactiae* found in both groups in this study conflicts with previous research in other countries (6); this may be due to the low rates of animal transport and commercialisation in EG, and the low prevalence in native animals that are isolated from other sources of infection. Furthermore, because the production sheep may have been exposed to good hygiene conditions in their original countries, the disease appeared to be controlled in these animals.

The population of isolated native animals studied was small when compared with the total sample size (35 of 1,002 animals). This was because these native animals were the only ones identified that had never had any contact with imported animals, and therefore reflected the situation of the country before the development of a broader sheep industry. In these native animals, the prevalence of *B. ovis* and *M. agalactiae* infection was much greater than that found in production flocks. This represents a serious risk for the sheep industry implementation and extension programme that is currently being conducted in EG, as has already been described in other countries (13). Interestingly, the initial concern was the reverse: that imported animals could act as carriers of diseases that were not present in native EG flocks.

Conclusion

Sheep from EG present high levels of infection with *C. pseudotuberculosis* and *C. abortus*, with seroprevalence values of 47.56 and 54.28%, respectively. The prevalence of specific antibodies against *M. agalactiae* and *B. ovis* in isolated native animals reveals that the diseases caused by these bacterial pathogens must be considered when implementing hygiene and preventive management procedures for animals that are being imported into the country.

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Résumé français: titre

Résumé

Mots-clés

Resumen español: título

Resumen

Palabras clave

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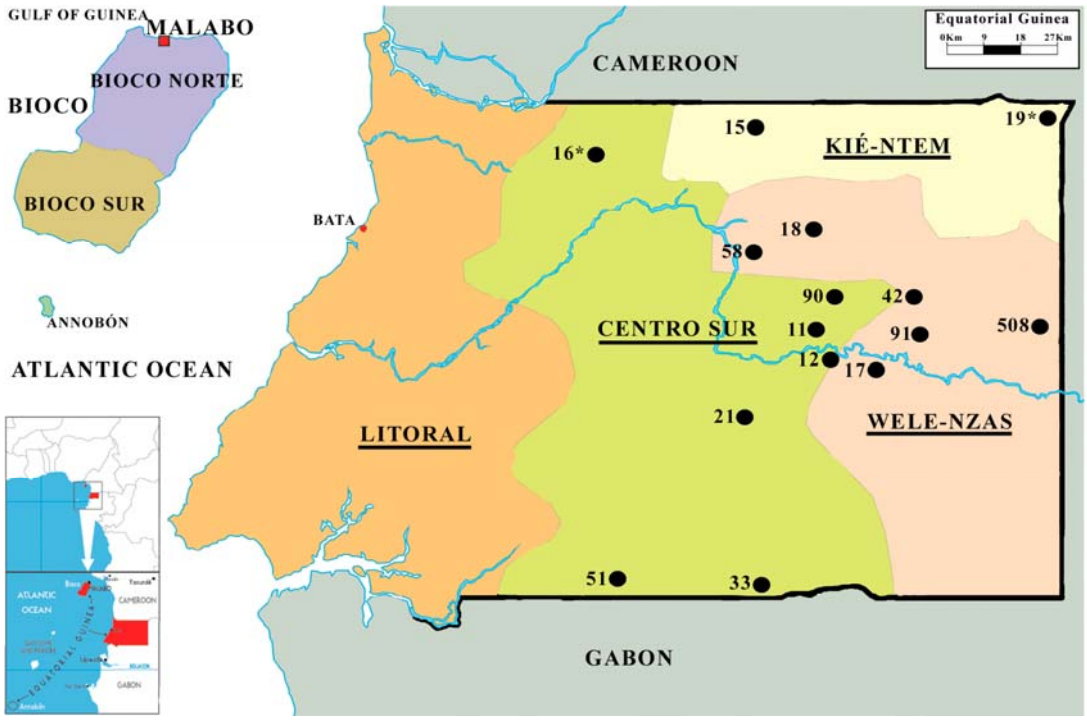

Fig. 1

Map of Equatorial Guinea showing the division of the country into six provinces, two in the insular region (Bioco Norte and Bioco Sur) and four in the continental region (Litoral, Centro Sur, Wele-Nzas and Kié-Ntem)

The names of the provinces are underlined, and the cities in which ovine serum samples that were included in this study were collected are represented with black dots; '*n*' indicates the number of production flock animals and '*n**' indicates the number of native sheep that were sampled in each location. The numbers beside each black dot represent how many production flock animals were sampled in each location, and the numbers followed by the symbol '*' represent the quantity of isolated native animals that were sampled in two different locations

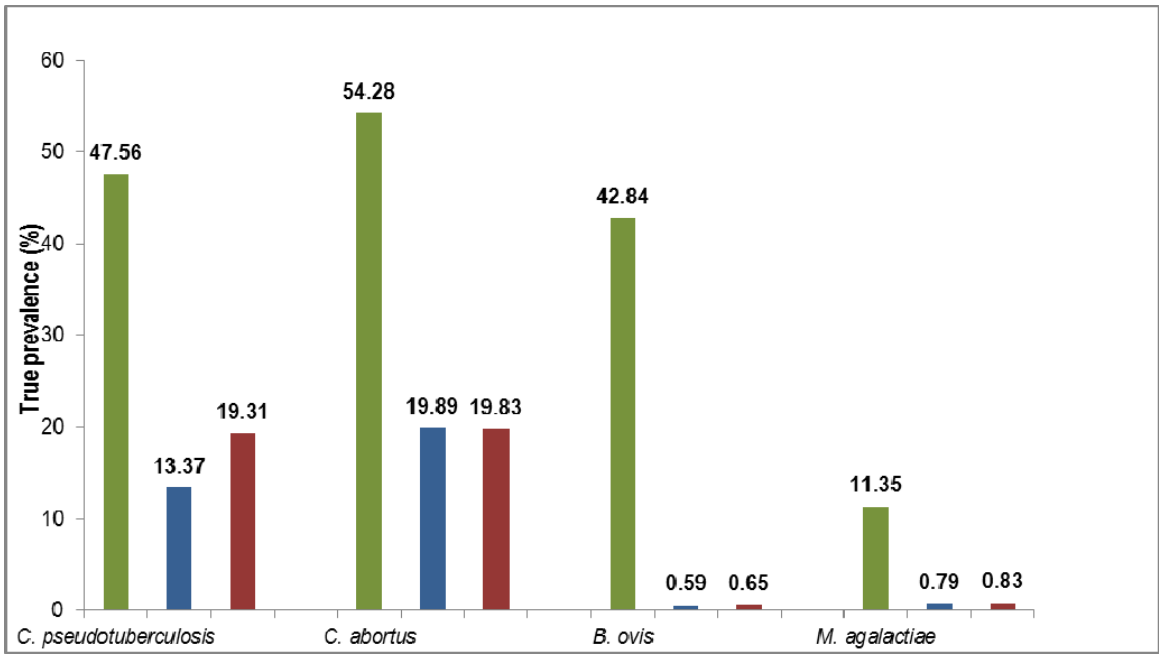

Fig. 2
True prevalence of specific antibodies against bacterial pathogens in isolated native sheep and production flock animals from Equatorial Guinea

The prevalence values were adjusted as proposed in (12), and the true prevalence estimates are shown at the top of the bars as percentages (%)

Isolated native animals (*n* = 35)

Production animals (*n* = 967)

Total (*n* = 1,002)