Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia

F. Ashenafi (1), S. Teshale (1), G. Ejeta (1), R. Fikru (1) & Y. Laikemariam (2)

(1) Faculty of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia. E-mail: teshalesori2002@yahoo.com
(2) National Animal Health Research Center, Sebeta, Ethiopia

Submitted for publication: 17 October 2006
Accepted for publication: 22 February 2007

Summary
A cross-sectional study was conducted in the pastoral region of Afar, in eastern and central Ethiopia, to determine the distribution of brucellosis in small ruminants. Between December 2005 and June 2006, 1,568 serum samples were taken: 563 samples from sheep and 1,005 from goats. One hundred and forty-seven of these (9.4%) tested positive using the Rose Bengal plate test (RBPT), and 76 (4.8%) also tested positive by the complement fixation test (CFT). Brucellosis was detected in all five administrative zones of the region. The difference in prevalence (P) among the zones was not statistically significant (P > 0.05). The seroprevalence of Brucella infection was found to be 5.8% (n = 58) in goats and 3.2% (n = 18) in sheep. A prevalence rate of 5.3% was observed in adult animals and 1.6% in younger sheep and goats. Caprine species (χ² = 5.56) and adult goats and sheep (χ² = 4.84) were found to be at higher risk of Brucella infection (P < 0.05). No statistically significant difference was found between males and females (χ² = 2.57, P > 0.05).

The study showed that small-ruminant brucellosis is a widely distributed disease in Afar. The authors recommend the implementation of well-organised disease control and prevention methods to mitigate the economic losses and public health hazard caused by the disease.

Keywords

Introduction
Goats and sheep are important domestic animals in tropical livestock production systems in Africa (4, 12), accounting for 21% of the global small ruminant population. Small ruminants fulfill a number of economic and social functions. According to statistics from the Central Statistical Agency (3), Ethiopia has over 18 million head of sheep and 24 million goats. Twenty-five percent of the sheep and 73% of the national goat population inhabit the lowlands (mostly pastoral areas) (16). Most goat populations in Ethiopia are raised under pastoral conditions. These small ruminants and their milk/meat products represent an important export commodity, which significantly contributes to the national economy.

However, although small ruminants represent a huge resource, production from this important asset does not realise its full potential, due to a number of technical and non-technical factors (11). First among the many factors which limit the economic returns from small ruminants is disease. One infectious disease which particularly impedes international trade is brucellosis. Brucellosis in small ruminants is mainly caused by Brucella melitensis and B. ovis. This disease is mainly characterised by abortion, with the development of yellowish, sticky layers on the
placenta in females. In male animals, it causes orchitis and epididymitis, as well as inflammation of the joints and bursae. The consequences of brucellosis in small ruminants are:
− infertility
− a high mortality rate in lambs and kids
− mastitis
− reduced milk production (18, 22).

Small-ruminant brucellosis has been shown to occur worldwide and is principally found in:
− Mediterranean countries
− the Middle East
− Africa
− India
− China
− Mexico
− parts of Latin America (13, 23).

The prevalence of brucellosis has been well studied in many of these regions and Table 1 lists the prevalence in sheep and goats in selected countries.

In Ethiopia, few studies have been conducted on brucellosis in small ruminants. Tekelye and Kasali (24) reported prevalence proportions of 1.5% in sheep and 1.3% in goats in the central highlands. Yibeltal (29) recorded prevalence proportions of 15% in sheep and 16.5% in goats in the Afar region and 1.6% in sheep and 1.7% in goats in the Somali region (Table I). The presence of this disease has also been reported in the Southern Nations, Nationalities and Peoples’ Regional State and pastoral areas of Borana (25). However, in general, the status of small-ruminant brucellosis in Ethiopia is not well studied. Yibeltal (29) observed that the prevalence of small-ruminant brucellosis was much higher in the Afar region, where farmers practise the communal use of grazing land, than in the Somali region, where clan-based flock/herd segregation is common. Thus, the practice of flock or herd mixing may have an impact on the distribution of brucellosis in Afar. The purpose of this study is to determine the influence of this method of husbandry on the distribution of small-ruminant brucellosis in this region.

### Materials and methods

#### Description of study areas

A cross-sectional study of small-ruminant brucellosis was conducted from December 2005 to June 2006 in Afar, which is inhabited by 1.3 million nomadic people. Afar is administratively divided into five zones (known simply as zone 1, zone 2, etc.), 33 districts and 353 peasant associations. Two of these districts were undergoing some administrative conflict and so were excluded from the study. A total of 18 districts were selected from the remaining 31. Out of these 18 districts, 38 peasant associations were randomly selected by lottery.

The Afar region has an annual rainfall of 516 mm on the western edge of the escarpment and 225.3 mm on the lava plain and volcano ash areas. The minimum and maximum temperatures are 18°C and 35°C, respectively. The altitude of the region ranges from 150 m below sea level to 1,000 m above. Afar has two international boundaries with Djibouti and Eritrea, and four national boundaries with the neighbouring states of Amhara, Oromiya, Somali and Tigray (Fig. 1). The main economic activity in the region is pastoralism, with regular movements across national and international boundaries.

Afar was selected for study for the following reasons:
− a high concentration of small ruminants (especially goats)
− the importance of the export trade in small ruminants and their products to this area
− husbandry practices that allow the mixing of flocks of different origin at communal watering and grazing areas
− the close permanent contact between humans and animals in this nomadic society.

The dominant animal species, based on population size, is cattle, followed by small ruminants. Both are used to generate income and as sources of milk and meat. There is a tendency to shift to the production of browsing species (small ruminants), due to the increasing human

### Table I

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep</td>
<td>Goats</td>
<td></td>
</tr>
<tr>
<td>Morocco</td>
<td>1.6%</td>
<td>4.1%</td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td>4.0%</td>
<td>18.0%</td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>8.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>2.4%</td>
<td>8.2%</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>3.0%</td>
<td>3.0%</td>
<td></td>
</tr>
<tr>
<td>Khartoum region of Sudan</td>
<td>14.2%</td>
<td>16.7%</td>
<td></td>
</tr>
<tr>
<td>Kenya</td>
<td>6.01%</td>
<td>6.01%</td>
<td></td>
</tr>
<tr>
<td>Somalia</td>
<td>7.2%</td>
<td>5.29%</td>
<td></td>
</tr>
<tr>
<td>Eritrea</td>
<td>1.4%</td>
<td>3.8%</td>
<td></td>
</tr>
<tr>
<td>Afar region of Ethiopia</td>
<td>15%</td>
<td>16.5%</td>
<td></td>
</tr>
<tr>
<td>Somali region of Ethiopia</td>
<td>1.6%</td>
<td>1.7%</td>
<td></td>
</tr>
</tbody>
</table>
population and shrinkage of grazing land, aggravated by the continued drought (7). Small ruminants from this region are an important export commodity to the Middle East.

**Study animals**

Afar has 953,000 sheep and 2.14 million goats. They are managed mainly under the pastoral production system, mixed with other species (such as cattle, camels, horses, etc.). The animals under study comprised the indigenous Afar goat (also known as the Adal, Assaorta, Denakil or Abyssinian short-eared goat) and Afar sheep (also called the Adal or Denakil sheep, from the group known as ‘fat-tailed hair sheep’). This and further information is detailed on the domestic animal genetic resources information system (DAGRIS) (12). No *Brucella* vaccine has been used in the study area.

**Study design**

The sample size was determined using a method recommended by Putt et al. (17). Accordingly, the estimated sample size was 1,344 animals, based on the expected brucellosis prevalence of 16% from previous preliminary reports, a precision level of 95% and the total number of small ruminants in Afar. However, to increase precision, 1,568 animals (sheep and goats) were included in this study. To determine the sampling unit, a two-stage cluster sampling at different hierarchical levels was used. Peasant associations and villages/flocks were used as the primary and secondary sampling units, respectively. The sampling frame consisted of a list of 353 peasant associations in Afar, obtained from the epidemiology unit of the Federal Ministry of Agriculture. Thirty-eight peasant associations from 31 districts were randomly selected by a lottery system. Peasant associations drawn in the lottery that were found to be inaccessible were replaced by other peasant associations which had a similar agro-ecology but were more accessible.

Villages and/or flocks were randomly selected during the field surveys. Flocks were considered as cluster units. Every sheep and goat above six months of age in a flock was sampled. A total of 1,568 sera (563 sheep and 1,005 goats) were collected, following standard procedures for detecting antibodies against *Brucella*. Approximately 8 ml of blood was collected from the jugular vein for serological examination, using plain vacutainer tubes and needles. The tubes were labelled and left tilted overnight at room temperature to allow for clotting. Next morning, sera
were removed from the clots by siphoning them into sterile cryovials. The sera samples were then shipped to the National Animal Health Research Center (NAHRC) laboratory, Sebeta, in an icebox and stored at –20°C, until the serological tests could be undertaken.

Serological tests

At the NAHRC, the Rose Bengal plate test (RBPT) was used to screen the serum samples to detect the presence of *Brucella* agglutinins. All samples testing positive by the RBPT were confirmed by retesting with the complement fixation test (CFT) at the National Veterinary Institute laboratory, Debre Zeit, Ethiopia.

**Rose Bengal plate test**

All serum samples collected were screened using the RBPT, according to the procedures described by Alton et al. (1) and the World Organisation for Animal Health (OIE) (28). The antigen used was Rose Bengal antigen, which constitutes a suspension of *B. abortus* (obtained from the Institut Pourquier, Montpellier, France). In brief, 30 µl of serum was mixed with an equal volume of antigen suspension on a glass plate and agitated. After four minutes of rocking, any visible agglutination was considered as positive (28). Agglutinations were recorded as 0, +, ++ and ++++, according to the degree of agglutination (14). A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. Those samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

**Complement fixation test**

All sera which tested positive by the RBPT were further tested, using the CFT, for confirmation. Standard *B. abortus* antigen for CFT (from the Veterinary Laboratories Agency, Addlestone, United Kingdom) was used to detect the presence of anti-*Brucella* antibodies in the sera. The control sera and complement were obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany. A haemolytic reaction of 25% at a dilution of 1:5 was considered the minimum positive threshold (5). The following sera were classified as positive (28):

- sera with a strong reaction of approximately 100% fixation of the complement (+), at a dilution of 1:5
- sera with about 75% fixation of the complement (3+), at a dilution of 1:10
- sera with about 50% fixation of the complement (2+), at a dilution of 1:20
- sera with about 25% fixation at a dilution of 1:40 (+).

Data analysis

The data collected in the field were entered into a computer on a Microsoft Excel spreadsheet. Statistical analysis (multivariate logistic regression) was performed using ‘Statistical package for the social sciences’ (SPSS), version 11.5 (for Windows). Categorical variables (species, sex, age and area) were expressed in percentages. The prevalence proportion was calculated as the number of animals testing positive by the RBPT/CFT, divided by the total number of animals tested. The association between each risk factor and the outcome variable was assessed using the Chi-square ($\chi^2$) test. For all analyses, a $p$-value of less than 0.05 was taken as significant.

Results

The results of this sero-survey for small-ruminant brucellosis in the Afar region are summarised in Table II. Of a total of 1,568 serum samples tested (563 sheep and 1,005 goats), 147 (9.4%) tested positive for brucellosis infection by the RBPT. Out of these 147 samples, 76 (4.8%; 95% confidence interval [CI]: 4.03, 5.57) also tested positive by CFT. Approximately 76.3% (n = 58) gave a result of at least 2+ and 60.5% (n = 46) tested at 4+ and 3+. Small-ruminant brucellosis was detected in all five zones of the Afar pastoral region, with zonal prevalence rates ranging from 2.3% in zone two to 6.7% in zone three. It was also shown that antibodies were detected in sheep and goats from the majority of the districts studied within each zone. Antibodies against *Brucella* were detected in sheep and goats raised in nearly 90% of the districts of Afar and 82% of the peasant associations.

The district level prevalence rates ranged from:
- 0% to 6.7% in zone one
- 1.7% to 3.4% in zone two
- 1.7% to 11.7% in zone three
- 2.4% to 7.1% in zone four
- 0% to 8.0% in zone five.

However, no statistically significant difference ($P > 0.05$) was observed in the prevalences among the districts and the five administrative zones of the Afar region.

This study showed a higher seroprevalence (by CFT) of brucellosis in goats than in sheep (Table III). The prevalence was 5.8% in goats (95% CI: 5.47 to 6.13) and 3.2% (95% CI: 1.89 to 4.51) in sheep, with a statistically significant difference ($\chi^2 = 5.56, P < 0.05$). Similarly, the prevalence of brucellosis in small ruminants was found to be higher in the adult animals of both species than in the younger ones. A seroprevalence of 5.3% (95% CI: 4.7 to
5.9) was observed in adult goats and sheep, while only 1.6% (95% CI: 0.95 to 2.25) of sera from young animals gave positive results (Table III). This difference in seroprevalence between the age groups was statistically significant ($\chi^2 = 4.84, P < 0.05$). However, no statistically significant difference ($\chi^2 = 2.57, P > 0.05$) was observed in the prevalence of brucellosis between male and female animals.

### Discussion

This study demonstrated that the overall seroprevalence proportion of small-ruminant brucellosis in Afar was 9.37% by the RBPT and 4.8% by CFT. Of the sheep and goats that tested positive by CFT, 76.3% gave reactions of 2+ and above, and 60.5% gave very strong reactions (4+ and 3+). Since none of the animals under study was vaccinated, this seems to reveal a moderate prevalence and natural transmission of *Brucella* organisms in the study area. Almost half of the sera which tested positive for anti-*Brucella* antibodies by RBPT, tested negative by CFT. This could be due to cross-reactions between *Brucella* and other bacteria which share similar epitopes.

The 4.8% prevalence of brucellosis in small ruminants observed by CFT in this study is in accord with the results of many previous observations, including studies in Karnataka in India (21), in the United Arab Emirates (2), in imported sheep and goats in Yemen (20), in Kenya (26) and in Eritrea (15). However, the result in this study is lower than the result recorded by Yibeltal (29) in the same area, i.e. 16%. This difference could be due to differences in the sample size and the tests used. Yibeltal (29) used the enzyme-linked immunosorbent assay to detect antibodies against *Brucella*. The authors used the RBPT as a screening test and the CFT as the confirmatory test. The RBPT, which is based on *B. abortus* antigen, is less sensitive in detecting antibodies against *B. melitensis* (9). On the other hand, the
prevalence recorded during this study is higher than that reported by Tekelye and Kasali (24), who observed prevalences of 1.5% in sheep and 1.3% in goat in the central highlands of Ethiopia. It is possible that this is due to variations in animal management and production systems. Central Ethiopia is characterised by mixed farming, in which fewer animals are raised and they are raised separately, whereas in the Afar region, large numbers of different species of animals are raised on communal pastures and watering areas.

Small-ruminant brucellosis was found to be distributed among all five zones in the Afar pastoral region, and there was no significant difference in the prevalence proportion among the zones. The disease was also detected in the adult and young age groups. The disease was more pronounced among adult animals (5.3%) than in young animals (1.6%). This may be due to the fact that goats excrete the organism for a long period of time, unlike sheep. This reduces the potential for disease spread among sheep flocks (19). A significantly higher seroprevalence was observed in goats (5.8%) than in sheep (3.2%). This finding is in agreement with the reports of El-Ansary et al. (6), Omer et al. (15), Radostits et al. (19) and Yibeltal (29).

In statistical terms, a significantly higher seroprevalence was observed in goats (5.8%) than in sheep (3.2%). This finding is in agreement with the reports of El-Ansary et al. (6), Omer et al. (15), Radostits et al. (19) and Yibeltal (29). Goats are at higher risk of acquiring Brucella infection than sheep. This may be due to the greater susceptibility of goats to Brucella infection. It could also be partly due to the fact that goats excrete the organism for a long period of time, unlike sheep. This reduces the potential for disease spread among sheep flocks (19). A statistically significant difference was also recorded in the prevalence of brucellosis between adults and young animals. A higher prevalence was found in adult sheep and goats. It has been reported that brucellosis is essentially a disease of sexually mature animals (18). Sexually mature and pregnant animals are more prone to Brucella infection and brucellosis than sexually immature animals of either sex (19). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (27). This may result from the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organisms, tend to increase in concentration with age and sexual maturity (19).

No significant difference was observed in the prevalence of brucellosis between males and females. Even though it is difficult to draw a firm conclusion, due to the smaller sample of males, the lack of difference between the two sexes observed in this study contradicts the established facts. Hirsh and Zee (10) have reported that male animals are less susceptible to Brucella infection, due to the absence of erythritol. However, in support of the present findings,

---

### Table III

**Seroprevalence of small-ruminant brucellosis in Afar, from December 2005 to June 2006, according to age and sex of animal**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of sera tested</th>
<th>CFT-positive number (%) (CI)</th>
<th>( \chi^2 ) (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>563</td>
<td>18 (3.2) (1.89-4.51)</td>
<td>5.56 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Goats</td>
<td>1,005</td>
<td>58 (5.8) (5.47-6.13)</td>
<td>4.84 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>170</td>
<td>4 (2.4) (1.1-3.7)</td>
<td>2.57 (P &gt; 0.05)</td>
</tr>
<tr>
<td>Female</td>
<td>1,398</td>
<td>72 (5.2) (4.59-5.81)</td>
<td>4.84 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Age:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young*</td>
<td>187</td>
<td>3 (1.6) (0.95-2.25)</td>
<td>2.57 (P &gt; 0.05)</td>
</tr>
<tr>
<td>Adult</td>
<td>1,381</td>
<td>73 (5.3) (4.7-5.9)</td>
<td>4.84 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Total</td>
<td>1,568</td>
<td>76 (4.8) (4.03-5.57)</td>
<td>( \chi^2 ) (p-value)</td>
</tr>
</tbody>
</table>

* Male goats and sheep aged less than or equal to one year and female animals that had not yet given birth were included in the younger age group.

CFT: complement fixation test
CI: confidence interval of 95%
Yibeltal (29) has also reported no observable difference in the prevalence of brucellosis between male and female sheep and goats.

In conclusion, the serosurvey described in this study shows that brucellosis is a widespread and well-established infection among goats and sheep across all zones and districts of the Afar region. Traditional animal husbandry and management practices are thought to support the spread of brucellosis in the area. Brucellosis presents a significant impediment to the economic potential of the large population of small ruminants in Afar. Since small ruminants and their products are an important export commodity, detaining seropositive animals in quarantine has a negative economic impact. The reproductive wastage associated with brucellosis is another obstacle to optimal exploitation of the small ruminant sector.

The authors recommend further epidemiological studies and isolation and identification of the biotypes of Brucella responsible for infection in Ethiopia. Such investigations have important implications for the type of vaccine that should be used and when monitoring the efficacy of control programmes. Mass vaccination could reduce the incidence of this disease to a significantly low level.

Distribution de la brucellose chez les petits ruminants de la région pastorale d’Afar, Éthiopie orientale

F. Ashenafi, S. Teshale, G. Ejeta, R. Fikru & Y. Laikemariam

Résumé
Une étude transversale visant à déterminer la distribution de la brucellose chez les petits ruminants a été conduite à Afar, région pastorale s’étendant du centre à l’est de l’Éthiopie. Entre décembre 2005 et juin 2006, un total de 1 568 échantillons de sérum ont été prélevés, dont 563 sérums ovins et 1 005 sérums caprins. L’épreuve au rose Bengale sur plaque (RBPT) a révélé la présence d’anticorps dirigés contre Brucella dans 147 de ces sérums, dont 76 sont également révélés positifs à l’épreuve de fixation du complément (EFC). La brucellose a été détectée dans les cinq zones administratives que compte cette région. Il n’a pas été constaté de différence significative au plan statistique (P > 0,05) entre les taux de prévalence d’une zone à l’autre. La prévalence sérologique de l’infection à Brucella était de 5,8 % (n = 58) chez les caprins et de 3,2 % (n = 18) chez les ovins. La prévalence chez les ovins et les caprins adultes était de 5,3 %, et de 1,6 % chez les jeunes. Les caprins en général (χ² = 5,56) et les adultes, caprins et ovins confondus (χ² = 4,84) présentaient un risque plus élevé (P < 0,05). Aucune différence statistiquement significative n’a été constatée entre les mâles et les femelles au regard du risque d’infection (χ² = 2,57 ; P > 0,05).

Cette étude a révélé que la brucellose est une maladie largement répandue chez les petits ruminants de la région d’Afar. Les auteurs recommandent la mise en œuvre planifiée de méthodes de lutte et de prévention contre la brucellose afin de limiter les pertes économiques et le danger que cette maladie représente pour la santé publique.

Mots-clés
Distribución de la brucelosis de los pequeños rumiantes en la región pastoral de Afar, Etiopía oriental

F. Ashenafi, S. Teshale, G. Ejeta, R. Fikru & Y. Laikemarium

**Resumen**
Se realizó un estudio sectorial en la región de Afar, situada en Etiopía oriental y central, para determinar la distribución de la brucelosis de los pequeños rumiantes. Entre diciembre de 2005 y junio de 2006, se tomaron 1.568 muestras de suero en 563 ovejas y 1.005 cabras. Se detectaron anticuerpos contra la brucelosis en 147 (9,4%) animales con la prueba en placa de rosa de Bengala (RBPT) y también en 76 (4,8%) con la prueba de fijación del complemento (CFT). La brucelosis se detectó en las cinco zonas administrativas de la región. No se observaron diferencias estadísticas significativas en la prevalencia en las distintas zonas (P > 0,05). La seroprevalencia de la infección por *Brucella* resultó del 5,8% (n = 58) en cabras y el 3,2% (n = 18) en ovejas. La tasa de prevalencia observada en los animales adultos alcanzó el 5,3% y en las ovejas y cabras más jóvenes el 1,6%. Se encontró que la especie caprina (χ² = 5,56) y las cabras y ovejas adultas (χ² = 4,84) corrieron mayor riesgo de contraer una infección por *Brucella* (P < 0,05). No se observaron diferencias estadísticas significativas entre machos y hembras (χ² = 2,57; P > 0,05).

El estudio mostró que la brucelosis de los pequeños rumiantes está muy extendida en Afar. Los autores recomiendan la aplicación de métodos sistemáticos de control y prevención de la enfermedad a fin de reducir las pérdidas económicas y las amenazas para la salud pública que implica la enfermedad.

**Palabras clave**

**References**


