

## Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems

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### SUMMARY

Samples from 2427 cattle, 661 goats, 104 sheep, 98 camels and 82 horses were screened for brucella infections by the Rose Bengal Test and positive reactors confirmed by the complement fixation test. In cattle, the highest individual seroprevalence was in dairy herds kept under the intensive husbandry system, with an individual prevalence of 8·2% and unit (herd) seroprevalence of 35·9%. This was followed by the pastoral husbandry system in the Western Lowlands with 5·0% individual but a higher unit (vaccination site) prevalence of 46·1%. The lowest was in the mixed crop-livestock system in the Southern Highlands with individual 0·3% and unit (village) prevalence of 2·4%. In sheep and goats, no positive animals were detected in the mixed crop-livestock areas. In the Eastern Lowlands individual prevalences of 3·8% (goats) and 1·4% (sheep) and unit prevalence of 33·3% (goats) and 16·7% were found, while 14·3% of individual goats and 56·3% of the units in the Western Lowlands were positive. No positive horses were found. The present study documents the first serological evidence of *Brucella* spp. infection in camels (3·1%) in Eritrea.

### INTRODUCTION

For the newly independent State of Eritrea, livestock are regarded as one of the most important assets for developing its economy, with an estimated indigenous livestock resource of about 1·65 million tropical livestock units, 76·2% of which are cattle [1]. As one of the most important causes of abortion and infertility in cattle and other livestock, and a serious risk to human health, brucellosis impedes economic development in developing countries. In several developed countries, including the USA, the European

Union and Australia, brucellosis is a notifiable disease given top priority for elimination. Despite its eradication in several developed industrialized countries, brucellosis remains as one of the economically most important zoonotic diseases worldwide.

The prevalence of human and animal brucella infections in the newly independent State of Eritrea is not known, but the infection is reported to be widespread in neighbouring countries including Ethiopia [2, 3], Sudan [4–8] Djibouti [9], Saudi Arabia [10–15] and Yemen [16]. As movement of animals between Eritrea and the Sudan and between Eritrea and Ethiopia is widespread, it is more than likely that infections are also widespread in Eritrea.

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The presence of *Brucella* spp. in livestock in Eritrea was well documented during the 1940s and 1950s. Cilli [17] isolated *Brucella melitensis* from a local cheese from Agordat, in the Western Lowlands, and he later reported the first serological evidence of brucella infection in goats and sheep in the Highlands [18] and in the Eastern Lowlands [19] of Eritrea. Thereafter infections were detected in dairy cattle and in slaughtered beef [20], and the first case of abortion in indigenous cattle due to a *Brucella* species, described by the authors as intermediate between *Brucella melitensis* and *Brucella abortus*, was reported [21]. The disease was subsequently reported in horses, mules and donkeys [22]. These findings led to early introduction of a test-and-slaughter program of eradication in the dairy farms [23].

The military and political upheavals that followed the end of the Italian administration, culminating in the 30-year war of independence from Ethiopia between 1961 and 1991, meant that no meaningful information was available on the prevalence of brucella infections for over 40 years. Limited serological surveys carried out by Tekleghiorgis [24] and others conducted after the end of the war in 1991 (G. Taeme, Ministry of Agriculture, personal communication) suggest that brucella infection is endemic among the dairy cattle in Eritrea, but these studies do not allow any prevalence estimates to be inferred.

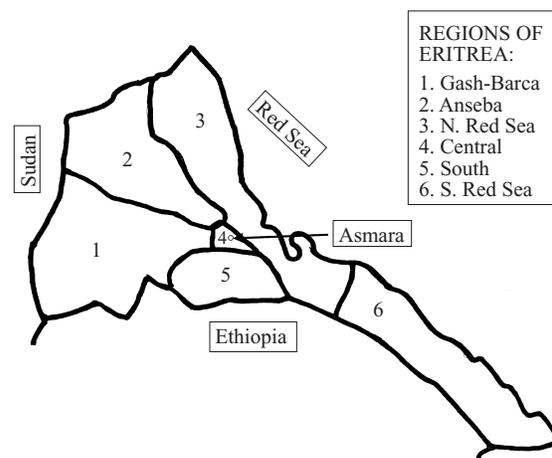
The aim of the present study was to describe the occurrence of brucella infections in cattle, sheep, goats, horses and camels in Eritrea in different areas and under various husbandry systems by investigating the prevalence of antibodies to *Brucella* spp.

## MATERIALS AND METHODS

### Study population and sampling frame

The study was conducted between July 1997 and November 1998. There is no vaccination against animal brucellosis in Eritrea, and thus all samples were from unvaccinated animals. Serum samples were collected from cattle, goats, sheep, camels and horses from five of the six administrative regions of Eritrea, representing three husbandry systems (Fig. 1):

- (1) intensive dairy farms in and around the capital city, Asmara;
- (2) extensive (traditional) pastoral husbandry systems in the Western and Eastern Lowlands;
- (3) extensive mixed crop-livestock systems in the Southern Highlands.



**Fig. 1.** Map of Eritrea showing administrative regions and sampling areas. Samples were collected from region 1 and 2 (Western Lowlands), 4 (Central Highlands), 5 (Southern Highland) and southern part of region 3 (Eastern Lowlands).

### Cattle

As it is widely accepted that sexually immature cattle are quite resistant to exposure to *B. abortus* and that susceptibility increases with sexual development and pregnancy [25], only pregnant heifers, cows and breeding males were sampled.

*Intensive dairy herds around Asmara.* Samples were collected from a reference population of 213, mainly Friesian or crossbred, dairy herds belonging to the Asmara Dairy Farmers' Association, the largest and oldest dairy association around the capital Asmara, with a total dairy cattle population of 4300 animals [1]. Only herds with 9 or more cows were included in the study. Of the 99 herds that satisfied this criterion, 72 farms were randomly selected to be included in the study. Serum samples were obtained from 1356 of 1807 adult animals in 64 herds, 8 herds having been removed from the study because of a reduction in size during the sampling period to below 9 cows or because the business was closed down. The size of herds under study ranged from 10 to 91 animals, with a median size of 23.5. Of the cattle tested, 1294 (95.4%) were females and 62 (4.6%) were males.

*Traditional husbandry systems (pastoral and crop-livestock).* Pastoralism is the predominant husbandry system in the Western and Eastern Lowlands of Eritrea. In the Western Lowlands, the predominantly female and relatively large herds graze a wide geographical area. The breed of cattle in this region is predominantly the local Zebu type, the *Begait* or *Barca*. The constant movement of these animals brings them into contact with large groups of cattle

Table 1. Prevalence of antibodies to *Brucella* spp. in cattle under different management systems and regions in Eritrea. Results from testing with Rose Bengal test (RBT) and complement fixation test (CFT)

Husbandry system	Administrative Region	Animals tested	RBT positive	RBT and CFT positive (%)	Units* tested	Number of units with $\geq 1$ animals RBT and CFT positive (%)	95 % confidence interval (%)
Intensive dairy herds	Asmara	1356	142	111 (8.2)	64	23 (35.9)	24.6–49.0
Mixed crop–livestock	South	342	2	1 (0.3)	41	1 (2.4)	0.1–14.4
Mixed crop–livestock	Anseba	208	2	1 (0.5)	11	1 (9.1)	0.5–42.9
Pastoral (Western lowlands)	Gash Barca	441	25	22 (5.0)	26	12 (46.1)	27.1–66.2
Pastoral (Eastern lowlands)	N. Red Sea	80	0	0 (0)	6	0 (0)	0–39.0

\* Units represent: herds in the intensive farms, villages in the crop–livestock system and vaccination sites in the pastoral systems.

and other livestock sharing the same grazing or watering places. In contrast, the herds in the Eastern Lowlands are fewer in numbers, smaller in size and graze in a smaller geographical area (Fig. 1). They are predominantly composed of another local Zebu breed, the *Arabo*.

Organizing a representative sample from a constantly moving pastoral population is difficult. However, pastoral cattle are regularly vaccinated against rinderpest. Thus we randomly selected some of these annual vaccination locations where large numbers of cattle are gathered as sampling sites. Serum samples were collected from 441 animals in 26 sampling (vaccination) sites in the Western Lowlands, and from 80 animals in 6 sites in the Eastern Lowlands. All animals in the flocks are gathered at these vaccination sites. No information on reproduction status was obtained from these sites.

In the crop–livestock system of the Southern Highlands, cattle, though used for milk, are mainly kept as draft animals and the herds contain a high proportion of males. The small local *Arado* breed of Zebu cattle is predominant in this region. The grazing system in the Highlands is based on communal use of lands owned and managed by one village, the animals rarely moving to neighbouring villages except when draft animals are sold or hired. The village was chosen as a sampling unit and serum samples were collected from 550 cattle in 52 randomly selected villages.

#### Goats and sheep

Serum samples were collected from goats from 29 randomly selected annual vaccination sites and surrounding places, in the Southern Highlands, Western Lowlands and Eastern Lowlands. From each site, serum samples were collected from 15–20 adult female

goats. A total of 661 samples were collected. Samples of 104 sheep in 9 sampling sites were collected from the Highlands and the Eastern Lowlands. Because of difficult working conditions it was not possible to obtain real random sampling of goats and sheep.

#### Camels

Serum samples were collected from 98 camels in 2 sampling sites in the Eastern Lowlands. Camels are typically kept by pastoral groups with goats/sheep but no cattle present in the group.

#### Horses

Horses were present mainly in the intensive dairy farms around the capital, Asmara. These animals are used to transport milk to the dairy plant and for the transport of animal feed. Serum samples were collected from 48 of the 82 horses present in the 64 intensive dairy farms under investigation.

#### Collection and handling of blood samples

Blood was collected (7 ml) in evacuated silicone-coated tubes (Becton Dickinson, Cockeysville, NJ) from the caudal vein from cattle in intensive farms or the jugular vein in other animals. Blood samples were left overnight to clot and the sera sent to the Central Veterinary Laboratory, Asmara in iceboxes and stored at  $-20^{\circ}\text{C}$  until serological testing was undertaken.

#### Serological tests

##### Rose Bengal test (RBT)

The RBT test was carried out according to the method described by Alton [26] with *Brucella abortus* antigen

Table 2. Influence of herd size on herd prevalence of antibodies to *Brucella* spp. in the intensive dairy farms around Asmara. Herd size represented by the number of animals tested

Number of animals tested*	No. (%) of herds tested within range	No. (%) of CFT positive herds within range tested	95% confidence interval (%)
9–15	23 (35.9)	9 (39.1)	20.5–61.2
16–25	25 (39.1)	7 (28.0)	12.9–49.6
26–35	9 (14.1)	3 (33.3)	9.0–69.1
> 35	7 (10.9)	4 (57.1)	20.4–88.2

\* All pregnant heifers, cows and breeding males were tested.

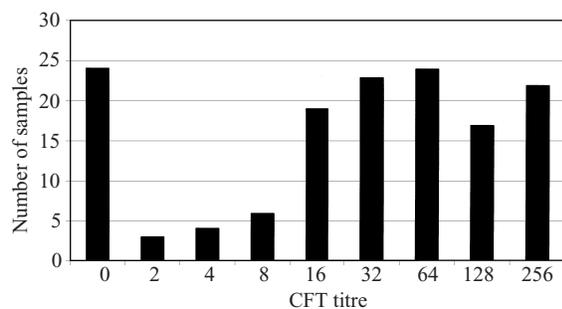


Fig. 2. Distribution of titre in the complement fixation test (CFT) in sera from 142 cattle positive on the Rose Bengal Test (RBT) from the intensive dairy herds around Asmara samples.

obtained from the Central Veterinary Laboratory (CVL), Weybridge, UK. Briefly, 25 µl of antigen were mixed, in flat plates, with an equal volume of bovine, camel or horse serum or with 75 µl of ovine or caprine serum.

#### Complement fixation test (CFT)

All the RBT-positive samples were re-tested by CFT using *Brucella abortus* (S99) antigen obtained from CVL. The CFT was performed according to the method of Alton [26] using cattle sera inactivated at 58 °C for 30 min and sera from sheep, goats and camels inactivated at 62 °C for 30 min. The tests were carried out in U-shaped wells of 96 well micro-titre plates (Bibby Sterlin, Stove, UK), using 25 µl of two-fold dilutions of inactivated sera, 1 in 10 dilution of S99 antigen, 5 IU of guinea pig complement (Sigma, Steinheim, Germany), and 3% of sensitized sheep red blood cells in veronal buffer (Sigma). Sera that showed anti-complementary activity were re-tested using a 5% bovine serum albumin (Sigma) in veronal buffer. The antibody titre of each serum was the highest dilution showing 25% or more fixation. Serum samples with antibody titres of 8 or higher were

regarded as positive; those with 4 were regarded as suspicious. A unit (herd, village, sampling site) was defined as positive if at least one animal was seropositive in both RBT and CFT. Reference negative and positive cattle sera supplied from CVL were used in all the serological tests.

#### Statistical analysis

Confidence intervals (95%) for cattle herd prevalences were calculated using EpiCalc 2000 (Jon Gilman and Mark Myatt, Brixton Books 1997). Because of clustering in units, no confidence intervals were calculated for individual prevalences.

## RESULTS

### Cattle

Of the 2427 bovine sera tested in all the regions under investigation, antibodies to *Brucella* spp. were detected in 135 (5.6%) by both RBT and CFT (Table 1). All but one of the seropositive cattle was female. A low seroprevalence was detected in cattle under the mixed crop-livestock husbandry system of the Southern Highlands, with an individual prevalence of 0.3% and unit (village) seroprevalence of 2.4%. In the dairy herds kept under the intensive husbandry systems of the farms around the capital, Asmara, the individual seroprevalence was 8.2% and the herd seroprevalence was 35.9%. No distinct trend was linked to herd size (Table 2). Cattle in the pastoral systems of the Western Lowlands had an individual prevalence of 5.0% and unit (vaccination site) seroprevalence of 46.1%.

The CFT titre was higher than 16 in 60.6% of all the RBT positive sera. The distribution of seropositive cattle according to their complement fixing antibody titres is depicted in Figure 2.

Table 3. Prevalence of antibodies to *Brucella* spp. in Eritrean goats, sheep, camels and horses from different husbandry systems

Husbandry system	Species	Region	Animals tested	RBT positive	RBT and CFT positive (%)	Units tested	Units with $\geq 1$ animal RBT and CFT+ (%)
Mixed crop–livestock	Goat	South	178	2	0 (0)	7	0 (0)
Pastoral (Western Lowlands)	Goat	Gash-Barca	323	18	14 (4.3)	16	9 (56.3)
Pastoral (Eastern Lowlands)	Goat	N. Red Sea	160	7	6 (3.8)	6	2 (33.3)
Mixed crop–livestock	Sheep	South	30	1	0 (0)	3	0 (0)
Pastoral (Eastern Lowlands)	Sheep	N. Red Sea	74	1	1 (1.4)	6	1 (16.7)
Pastoral (Eastern Lowlands)	Camel	N. Red Sea	98	3	3 (3.1)	3	1 (33.3)
Intensive dairy production	Horse	Asmara	82	0	0	40	0 (0)

### Goats, sheep, camels and horses

The results of the serological examination linked to the husbandry systems, collection sites and number of animals tested are presented in Table 3. The highest individual prevalence (4.3%) and unit prevalence (56.3%) in goats was recorded in the Western Lowlands; followed by the Eastern Lowlands with an individual prevalence of 3.8% and unit prevalence of 33.3% and with no detection of antibodies in serum samples obtained from the mixed crop–livestock villages in the Southern Highlands.

Similarly the individual prevalence was higher in sheep in the Eastern Lowlands, with individual prevalence of 1.4% and unit prevalence of 16.7%, than in samples obtained from the Southern Highlands (no positive samples). Three of 98 camels were seropositive, constituting an individual prevalence of 3.1% and unit prevalence of 33.3%. No antibodies to *Brucella* spp. were detected in any of the horse samples.

### DISCUSSION

The high individual prevalence of brucella seropositivity in cattle in the intensive dairy farms around Asmara and the Western Lowlands agree with the characteristics of these husbandry systems. According to FAO [27], the levels of brucella infections tend to be relatively high on intensive farms, whether these have indigenous cattle or introduced breeds. It has been reported that the risk of infection increases with the change from the purely extensive pastoralist (nomadic) to a more intensive form of cattle management [28]. The intensive dairy herds were composed mainly of imported or crossbred cattle, some of which might

have originated from infected sources. Most of the dairy farms were kept under poor management systems, characterized by overcrowding, poor hygiene and poor ventilation, and were very small by international standards (9–20 animals).

As the population of the capital city, Asmara, is supplied with milk and milk products (cheese, yoghurt and butter) from the dairy farms with high seroprevalence, there is an urgent need to reduce the prevalence of the infection in these farms. This would require a clear understanding of risk factors for introduction of infection to a herd. For example, it has been reported that one of the problems of controlling brucellosis in the Republic of Ireland was the tendency for the disease to spread to adjoining farms [29]. The farms around Asmara are close to each other, but the present study did not investigate whether or not there have been regular contacts between farms. It has also been speculated that use of common grounds for grazing different herds could be a potential problem in maintaining disease-free status and that community pastures have been implicated as risk factors for the transmission of the disease [29–31].

The high seroprevalence in the extensive pastoral system in the Western Lowlands may be largely attributed to the mobile nature of these herds and their increased opportunity to come into contact with other, potentially infected, herds during their movement into the different parts of the region. Migrations increase the chances of coming into contact with geographically limited or seasonally abundant disease and also increase the opportunity for interaction of domestic and wild animals, which facilitate transmission of disease [32]. The lowest seroprevalence was in the herds of cattle in the mixed crop–livestock husbandry system of the Highlands where cattle herds

are sedentary, with little contact between the villages and frequent contact between cattle of the same village.

The seroprevalence observed in cattle in our study is similar to figures reported from countries with similar management systems. For example, Rikin [33] reported a seroprevalence of 7.1–8.6% in unvaccinated nomadic herds in Nigeria, and as high as 26% in cattle concentrated in watering points. Hellmann [7] reported a seroprevalence of 6.5% among Dinka cattle in southern Sudan, but found a seroprevalence of 22.5% among Fellata cattle in the same region. McDermott [34] reported a seroprevalence of 21.7% among Dinka cattle in a different locality of Southern Sudan. Kadohira [35] studied seroprevalence in a pastoral area, tropical highland area and tropical coastal area in Kenya. Though there were variations by farm, area and district, the authors reported that the highest individual seroprevalence of 15% was in the pastoral area and the lower of 2% was in the tropical highland area.

Because the number of goats and sheep tested was small and we used non-random sampling, it is not possible to make firm conclusion from the lack of detection of antibodies from the caprine or ovine sera obtained from the mixed crop-livestock system of the Highlands. However, the seroprevalence of goats in pastoral regions is in agreement with a survey conducted by Cilli [19] nearly 50 years ago. It is interesting to note that Cilli [18] found a very low seroprevalence in sheep and goats of the Southern Highlands (0.3%, and 1.9% respectively), and that Cilli [22] also reported that 23 of 157 (14.6%) horses, donkeys and mules tested were seropositive. The present study documents for the first time the presence of brucella antibodies in Eritrean camels, but further studies are required to establish the seroprevalence in camels in the different geographical areas of the State of Eritrea.

CFT has a high specificity and is considered to be the nearest approach to a definitive test for brucella infection [36–38]. In non-vaccinated population as in Eritrea, the specificity is supposed to be 100% [37]. Corbel [39] maintains that serological cross-reactions produced by other organisms tend to be of little importance until the prevalence of the disease falls to a very low level.

In summary our results demonstrate that brucella infections are widely spread in domestic animals under intensive management as seen in the dairy herds around Asmara and in the large pastoral herds in the

lowlands. With the high seroprevalence in the dairy herds, but a low seroprevalence in traditional herds in the same region, it may be possible to reduce the infection pressure by actions directed mainly at the commercial dairy herds. Economical and management systems linked to these herds may facilitate such a programme. The prevalence of brucella infections in man in Eritrea is unknown, but the disease is considered to be common. Further clinical and epidemiological studies may reveal more about the role of domestic animals as a source of infection to man.

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