Reliability of results of the Rose Bengal test performed for export control in northern Somalia

F. Ostanello (1), L. Farina (2), C. Turilli (2), P. Serra (3), V. Cagnolati (4), M. Abdullahi (5), A. Scagliarini (1) & S. Prosperi (1)

(1) Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Via Tolara di Sopra 50, 40064 Ozzano Emilia (Bologna), Italy
(2) Istituto Zooprofilattico Sperimentale delle Venezie, Via Romea 14/A, 35020 Legnaro (Padua), Italy
(3) Coordinatore Progetti Veterinari in Somalia, Cooperazione Internazionale, Azienda Unità Sanitaria Locale Città di Bologna, Via Gramsci 12, 40100 Bologna, Italy
(4) Rappresentante per l'Africa Orientale - Terra Nuova, P.O. Box 74916, Nairobi, Kenya
(5) Director General, Ministry of Livestock, Hargeisa, Somaliland

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Summary
Sera from sheep and goats in northern Somalia which are exported to countries of the Persian Gulf are systematically checked for brucellosis by local veterinary teams. The standard test used is rapid seroagglutination using the Rose Bengal test (RBT) and seropositive animals are not exported. In order to assess the reliability of the serological results, three randomised batches of samples (653 sera), corresponding to an equivalent number of shipments (October and December 1994 and March 1995) were counterchecked. Control RBTs were carried out by expatriate veterinarians working on behalf of international non-governmental organisations and by the Istituto Zooprofilattico Sperimentale di Padua, Italy, which also performed the complement fixation test (CFT). A fourth batch (n = 100), including a group of sera found positive by the local veterinary teams, was also checked. Agreement ranged from 96.3% to 98.5%.

Keywords

Introduction
Of the countries of Africa, Somalia is one of the most important in terms of exports to the Middle East. For instance, a considerable number of sheep and goats are shipped from Berbera (north-west Somalia) to the countries of the Persian Gulf, especially to Saudi Arabia. Figures provided by the local veterinary authorities describe an average livestock export flow of 2.5 million per year during the period between 1994 and 1996. The health authorities of Saudi Arabia request certification for each flock under export, stating Brucella-free status, for slaughtering and local consumption. Therefore, all sheep and goats must be individually tested for brucellosis before shipment, using the Rose Bengal test (RBT).

The aim of the study was to undertake a comparative evaluation of quality performances for RBTs of the local veterinary group and of the expatriate group (veterinarians working in international non-governmental organisations), compared to that of the Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy (IZS-Padua), used as the ‘gold standard’.

Materials and methods

Serum samples
Three randomised batches of samples (A, B and C), consisting of 653 sera, were obtained on 28 October and 13 December 1994 and 19 March 1995, respectively (Table I).

The number of samples was chosen to be 95% representative of the total number of sheep and goats exported through the Berbera port during the months when sampling was performed; therefore a 2.8 ± 1.3 true prevalence was taken into account as a hypothetical base of brucellosis infection.
The true prevalence has been computed (13) through the apparent prevalence determined in a previous study (12), adjusted for sensitivity and specificity to RBT of 0.86 and 0.99, respectively (3, 6, 9).

A fourth batch (D) of 100 sera, obtained on 19 March 1995, from sheep and goats found positive to RBT by the local veterinary groups in Somalia, was also examined (Table I). All the animals examined were between one and two years of age.

Since the enforcement of the Siad Barre regime, a ban has been imposed on the export of female livestock, therefore only males could be used for this research.

Serological examinations

Under field conditions, local veterinary groups in Somalia perform the RBT on all animals in the flock for export. However, in this study, the expatriate veterinarians took the sera from each animal randomly selected for RBT and carried out the RBT under blind conditions. Subsequently, sera were frozen at -20°C and then shipped to the Serological Unit, IZS-Padua, Italy, whilst ensuring maintenance of the cold chain. The RBT and the complement fixation test (CFT) were then performed under standard laboratory conditions. The recommended standard methodologies were followed during the execution of the RBTs (11). Only one type of antigen (produced by the Istituto Zooprofilattico Sperimentale dell’Abruzzo e Molise in Teramo, Italy) was used. This antigen was standardised against the international standard anti-B. abortus serum (ISaBS) to give a positive reaction at a dilution of 1:47.5, according to European Union (EU) requirements (3). Both the expatriate team and the Serological Unit of the IZS-Padua, Italy, whilst ensuring maintenance of the cold chain. The RBT and the complement fixation test (CFT) were then performed under standard laboratory conditions.

The complement fixation antigen (produced by the Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia, Brescia, Italy), was standardised against the ISaBS according to EU regulations, to produce 50% inhibition of haemolysis with a 1:200 dilution of ISaBS (3). The CFT was performed with a warm fixation microtechnique, using two 100% haemolytic units of complement and two 100% haemolytic units of haemolysin. The CFT results were expressed through complement fixation test international units (CFTIU). The threshold titre was fixed at ≥ 20 CFTIU.

Statistical analysis

Cochran’s Q test was utilised in order to evaluate the total extent of positive/negative sera within the three batches of RBT tests. Cochran’s Q test represents a development of McNemar’s chi-square test for matched data, since it shows whether a variation in the proportion of the different categories of results (positive/negative) takes place among the three batches of serological tests (13).

If conflicting data were ≥ 10, the results obtained through RBT performed by each team were compared to those of the other groups by means of McNemar’s chi-square test for matched data.

Similarly, RBT performed respectively by the local veterinary groups and the expatriate veterinarians, was compared to RBT obtained by IZS-Padua. This laboratory utilised CFT and RBT through parallel and/or serial matching, and all the above data were analysed through McNemar’s chi-square test for matched data if conflicting data were ≥ 10.

Finally, the values of observed proportion agreement and of chance proportion agreement (both negative) were computed. The significance level chosen was 1% (p < 0.01).

Results

Values from each group regarding apparent prevalence detected by each laboratory are reported in Table II.

Groups A, B and C

Cochran’s Q test highlighted statistically significant differences in matching the proportion of positive animals to the three RBTs (Q = 16.75; p = 0.0002). Such a variation persisted when the results from the local veterinary groups in Somalia were compared with those from the IZS-Padua (χ² = 10.23; p = 0.0009) (Fig. 1), whereas the same variation was not observed when the results from the expatriate veterinarians were matched with those from the local technicians (χ² = 5.26; p = 0.0192) (Fig. 1). However, when comparing the proportion of positive animals resulting from the RBT by local groups and the RBT and CFT parallel testing, the outcome difference did not appear statistically significant (χ² = 4.76; p = 0.027) (Fig. 2).
Table II
APPARENT PREVALENCE OF BRUCELLA INFECTION DETECTED BY EACH LABORATORY

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Rose Bengal test Local veterinary groups in Somalia</th>
<th>Rose Bengal test Expatriate veterinarians in non-governmental organisations</th>
<th>Rose Bengal test Istituto Zooprofilattico Sperimentale delle Venezie, Padua</th>
<th>Complement fixation test Istituto Zooprofilattico Sperimentale delle Venezie, Padua</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 164)</td>
<td>Positives 6 3.7 95% CI 1.5-8.1</td>
<td>Positives 4 2.4 95% CI 0.8-6.5</td>
<td>Positives 3 1.8 95% CI 0.5-5.7</td>
<td>Positives 3 1.8 95% CI 0.5-5.7</td>
</tr>
<tr>
<td>B (n = 166)</td>
<td>Positives 6 3.6 95% CI 1.5-8.1</td>
<td>Positives 7 4.2 95% CI 1.9-8.8</td>
<td>Positives 3 1.8 95% CI 0.5-5.7</td>
<td>Positives 4 2.4 95% CI 0.8-6.45</td>
</tr>
<tr>
<td>C (n = 323)</td>
<td>Positives 14 4.3 95% CI 2.5-7.3</td>
<td>Positives 4 1.2 95% CI 0.4-3.4</td>
<td>Positives 4 1.2 95% CI 0.4-3.4</td>
<td>Positives 6 1.9 95% CI 0.8-4.2</td>
</tr>
<tr>
<td>A+B+C (n = 653)</td>
<td>Positives 26 4.0 95% CI 2.7-5.8</td>
<td>Positives 15 2.3 95% CI 1.3-3.6</td>
<td>Positives 10 1.5 95% CI 0.8-2.9</td>
<td>Positives 13 2.0 95% CI 1.3-3.5</td>
</tr>
<tr>
<td>D (n = 100)</td>
<td>Positives 100 – 95% CI –</td>
<td>Positives 85 – 95% CI –</td>
<td>Positives 80 – 95% CI –</td>
<td>Positives 78 – 95% CI –</td>
</tr>
</tbody>
</table>

AP : apparent prevalence
CI : confidence interval

When comparing the proportion of positive animals resulting from the RBT by the local veterinary groups and the RBT and CFT serial testing, the outcome difference appears to be statistically significant ($\chi^2 = 12.04; p = 0.0003$) (Fig. 2).

When comparing the proportion of positive animals resulting from the RBT by expatriate veterinarians and the RBT and CFT serial testing, the outcome difference does not appear to be statistically significant ($\chi^2 = 0.00; p = 1$) (Fig. 2).

**Discussion and conclusion**

The apparent prevalence of Brucella infection in sheep and goats seems to be similar to that reported by other authors (2, 4), even though only young males were examined in the present study (Table II). Furthermore, the prevalence values confirm the validity of the sampling method used, in that the results were very similar to those previously found by Serra et al., who analysed almost 50% of the exported sheep and goats from northern Somalia in the period from September 1994 to January 1995 (12).

It should be noted that the lowest apparent prevalence values were recorded at the Serology Unit of the IZS-Padua, utilising RBT. However, the results of the RBT performed by local veterinary professionals in Somalia and expatriate veterinarians do not differ significantly.

The statistically significant difference between the RBT results of local veterinarians and IZS-Padua disappears if the comparison is performed utilising the results of RBT and CFT parallel testing. Such an interpretation results in an increase of the sensitivity and the predictive value of the result of a negative test, but reduces specificity and positive predictive value (5, 7, 10, 13).

The RBTs performed by the local veterinary groups indicate a certain number of animals as positive, whereas the same animals were found negative to RBT performed by IZS-Padua. In other words, a significant number of animals tested by IZS-Padua (empirical gold standard) and assigned to the group of negative animals were previously found to be positive by local veterinary groups (Table III).
The higher proportion of positive animals detected by local veterinary groups and expatriate veterinarians compared to those found by IZS-Padua may be due to a number of factors, including the following:

a) hot climate, causing an excessive evaporation of sera and antigen (8)

b) amount of antigen lower than the standard dose (0.03 ml per unit) (1)

c) too many samples analysed together resulting in a prolonged reading time (1)

d) storage and shipment of serum samples.

In addition, the local veterinarians in Somalia may prefer not to ship doubtful animals in order to minimise risks of rejection by importing countries in the event of randomly performed serological tests. This behaviour concerning RBT
Comparison of the results of Rose Bengal and complement fixation testing among the three laboratories (groups A, B and C)
reading may actually imply that a certain number of animals suitable for export are excluded because of the lack of the laboratory confidence.

The results obtained show that the laboratory quality standards of the local veterinary groups in Somalia can be considered equivalent to that of expatriate veterinary teams belonging to international organisations.

Fiabilité des résultats de l'épreuve au rose Bengale réalisée pour le contrôle des exportations au nord de la Somalie

F. Ostanello, L. Farina, C. Turilli, P. Serra, V. Cagnolati, M. Abdullahi, A. Scaglierini & S. Prosperi

Résumé
Les petits ruminants qui sont exportés du nord de la Somalie vers les pays du Golfe arabe sont systématiquement soumis à des tests de détection de la brucellose par des vétérinaires appartenant à des associations locales. Le test employé est l'épreuve d'agglutination sur lame au rose Bengale. Les animaux possédant des anticorps sont exclus de l'exportation. Dans le but d'évaluer la fiabilité des résultats ainsi obtenus, une équipe internationale de vétérinaires coopérants a réalisé sur place cette même épreuve sur des prélèvements (635 sérums) appartenant à trois échantillons aléatoires, représentatifs des lots exportés respectivement en octobre 1994, en décembre 1994 et en mars 1995. Les sérums étaient ensuite testés à l'Istituto Zooprofilattico Sperimentale delle Venezie (Padoue, Italie), afin de comparer les résultats. Une épreuve supplémentaire de contrôle, utilisant la fixation du complément, était réalisée dans ce même Institut. Un quatrième échantillon (n = 100), constitué par des sérums qui avaient donné des résultats positifs lors de l'épreuve effectuée par les vétérinaires locaux, a été contrôlée de la même manière. L'indice de concordance variait de 96,3 % à 98,5 %.

Mots-clés

Fiabilidad de los resultados obtenidos con la técnica del Rosa de Bengala llevada a cabo para controlar las exportaciones en el norte de Somalia

F. Ostanello, L. Farina, C. Turilli, P. Serra, V. Cagnolati, M. Abdullahi, A. Scaglierini & S. Prosperi

Resumen
Los ovinos y caprinos procedentes de la región septentrional de Somalia y destinados a la exportación hacia los países árabes del Golfo Pérsico son sistemáticamente sometidos a pruebas de detección de brucelosis, realizadas por veterinarios locales. La técnica utilizada para realizar el diagnóstico es la
seroaglutinación al Rosa de Bengala. Los animales que resultan positivos no son exportados. Con el objetivo de evaluar la fiabilidad de los resultados serológicos obtenidos, tres lotes de muestras (un total de 653 sueros) obtenidos aleatoriamente y representativos de las partidas exportadas durante los meses de octubre y diciembre 1994 y marzo 1995, han sido analizadas de nuevo in situ por veterinarios pertenecientes a organismos de cooperación internacional y luego en Italia por el Istituto Zooprofilattico Sperimentale delle Venezie (Padua) donde además se utilizó la fijación del complemento como prueba de control. Un cuarto lote (n = 100) proveniente del grupo de sueros considerados positivos por los veterinarios locales ha sido controlado de la misma manera. El grado de concordancia variaba del 96,3% al 98,5%.

**Palabras clave**

**References**