Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan

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Summary

Brucellosis was studied in 2,225 camels, 20 camel nomads and 33 abattoir workers in certain nomadic localities in Sudan, using serum and milk samples. Lymph nodes, testicular tissues and udder tissues from positive camels and hygroma aspirates from three affected cows were used for isolation of Brucella. Serum samples were examined by Rose Bengal plate test (RBPT), modified RBPT (mRBPT), serum agglutination test (SAT) and competitive enzyme-linked immunosorbent assay (cELISA), and milk by the milk ring test.

Overall seroprevalence in camels (milk and serum samples) was 37.5%. The seroprevalence in males was 28.2% and in females 40.1%. Twelve (60%) of the 20 nomads and three (9%) of the 33 abattoir workers had positive antibody titres. Brucella abortus biovar 6 was isolated from two camels and three cows. Two isolates, one from each species, were atypical. The bacteriological findings suggested that camels were infected from cattle, the primary hosts of B. abortus. The mRBPT was suitable for screening camel sera for brucellosis, but the cELISA detected 2.1% more positives. The SAT antibody concentrations ranged between <13 and 3,282 IU/ml.

Keywords

Brucella abortus biovar 6 – Brucellosis – Camel – Cattle – Human – Sudan.

Introduction

Many tribes in different parts of the Sudan depend entirely on camels for their livelihood. Camel meat is consumed throughout the country and the animals contribute effectively to the economy by their use in agricultural practices and exportation. However, brucellosis has emerged as a major cause of abortion, hence a constraint to their breeding (1, 2, 11), and has had a negative impact on the export of camels.

Reports from veterinary laboratories have indicated that the prevalence of brucellosis in camels in some localities in Sudan is increasing (7).

The aim of this work was to study the prevalence of brucellosis in camels and the people in contact with them, and in other animals, to compare different serological tests used for diagnosis of the disease and to identify which Brucella species affect camels and cattle. It was also intended to investigate the epidemiological factors responsible for the spread of the disease in certain nomadic areas of Sudan.
Materials and methods

Areas of study

Camels were examined randomly for brucellosis in common grazing fields (Fig. 1) and water points (Fig. 2) in four localities in Sudan, designated A, B, C and D.

Sampling

A total of 2,225 serum and milk samples were collected for the diagnosis of brucellosis from nomadic and slaughtered camels of different sexes and ages from six months to over 15 years of age. The samples were obtained from all or most of the camels in herds that were selected randomly for examination, or from camels brought to slaughter in the main abattoir in the area. Samples included 2,000 blood specimens for serum and 225 milk samples, in addition to lymph nodes, testicular and udder tissues obtained from serologically positive camels at slaughter, three bovine hygroma aspirates from cattle presented for slaughter, and human serum samples from 20 camel nomads and 33 abattoir workers.

Serological diagnosis of brucellosis

The 2,000 serum samples from camels were screened by the Rose Bengal plate test (RBPT) and re-examined by modified (m) RBPT as described by Blasco et al. (5). Of the RBPT-negative serum samples, 560 were selected randomly and re-examined by competitive enzyme-linked immunosorbent assay (cELISA) at the Central Veterinary Research Laboratories, Soba, Khartoum, Sudan. One hundred random samples from the RBPT-positive sera were re-evaluated by standardised serum agglutination test (SAT) (10) for the measurement of antibody concentrations. Any sample that contained 30 international units (IU)/ml or more on the SAT was considered positive, in accordance with the guidelines of the World Organisation for Animal Health (OIE) (14). The human serum samples were also examined for the disease with the two RBPTs. Each of the 225 milk samples was supplemented with 1 ml of brucellosis-negative bovine milk to add fat globules (12) and examined by the milk ring test (MRT) (10).

Bacteriological investigations

Preparation and examination of samples

Each lymph node, udder sample and testicular sample was homogenised with a sterilised pestle and mortar using sterilised sand and phosphate buffered saline (PBS). Milk samples were centrifuged at 3,000 revolutions per minute (rpm) for 5 min to obtain sediment and cream.

Tissue homogenates, milk cream, milk sediment, and the hygroma aspirates were used separately to prepare slide smears. After staining with a modified Ziehl–Neelsen (ZN) stain, the slides were examined microscopically for weak acid-fast organisms (6).

Bacteriological culture

Samples in which weak acid-fast organisms were detected were cultured on serum dextrose agar (SDA) or tryptose agar (TA) media without antibiotics (which were not affordable), incubated at 37°C in an atmosphere of 10% CO₂ using candle jars and examined daily for 10 days for growth of weak acid-fast bacteria. Samples slightly contaminated by other bacteria were purified by subsequent subculture. Heavily contaminated samples that contained weak acid-fast organisms were emulsified in PBS and used for inoculation of guinea pigs (1 ml suspension intramuscularly). The guinea pigs were euthanised after 21 to 35 days and their sera examined for Brucella antibodies using RBPT. Spleens from serologically positive guinea pigs were removed, macerated by sterile forceps, cultured on SDA or TA media, and examined in much the same way as the primary cultures.

Isolates were identified as Brucella according to the method of Corbel (6). Brucella isolates were also sent to the Food and Agriculture Organization/World Health Organization/OIE Collaborating Centre for Reference and Research on Brucellosis at the Veterinary Laboratories Agency in the United Kingdom to be identified at the species and biovar levels.
Results

Serological test results

Serological data from evaluation of samples using the RBPT, mRBPT, cELISA and MRT are presented in Table I. Of the 2,000 serum samples, 797 (39.9%) were positive by the RBPTs and 809 (40.5%) by the cELISA test. Of the 225 milk samples, 38 (16.9%) were positive by the MRT. Of the 2,225 samples collected, 489 were from males and 1,736 from females, with 138 (28.2%) and 697 (40.1%), respectively, testing positive. The overall prevalence was 37.5%. The prevalence of the disease in the four localities, A, B, C, and D, was 36.2%, 37.8%, 36.4%, and 45.5%, respectively. There were no differences between RBPT and mRBPT in terms of sensitivity. The agglutination reactions on the mRBPT occurred readily. Twelve (2.1%) of the 560 RBPT-negative samples were positive on the cELISA. Most of these cELISA-positive samples gave results only marginally above the negative cut-off values. When 100 RBPT-positive samples were evaluated using the SAT, all but one tested positive (antibody level > 30 IU/ml). Antibody titres on the SAT ranged from 30 to 100 (17%), 101 to 819 (69%), and 820 to 3,282 IU/ml (13%).

Bacteriological examinations

Mesenteric lymph nodes from one female camel, testicular tissues and mesenteric lymph nodes from one male camel, and hygroma aspirates from three cows yielded six Brucella isolates. The infected mesenteric lymph nodes and the testicles were congested and haemorrhagic (Fig. 3). All the Brucella isolates produced oxidase, catalase, urease and hydrogen sulphide, they were CO₂-independent and nitrate-positive, but negative for indole, methyl red, Vogues Proskaur citrate and glucose.

The results of the identification of the isolates to species and biovar levels are presented in Table II. All strains were of B. abortus biovar 6, but two strains were atypical isolates of B. abortus biovar 6 due to their sensitivity to thionine at 20 µg/ml.

Table I
Prevalence of brucellosis in camels in the four nomadic localities in Sudan

A total of 2,000 serum samples were examined by the Rose Bengal plate test (RBPT), modified RBPT and competitive enzyme-linked immunosorbent assay. One hundred RBPT-positive samples were tested by serum agglutination test. The milk samples were examined by the milk ring test.

<table>
<thead>
<tr>
<th>Age 6 months to 1 year</th>
<th>Number of serum samples</th>
<th>Age 1 to 3 years</th>
<th>Number of serum samples</th>
<th>Age &gt;3 to 15 years</th>
<th>Number of serum samples</th>
<th>Total number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested</td>
<td>Positive</td>
<td>Tested</td>
<td>Positive</td>
<td>Tested</td>
<td>Positive</td>
<td>Tested</td>
</tr>
<tr>
<td>401</td>
<td>56</td>
<td>176</td>
<td>35</td>
<td>1,423</td>
<td>706</td>
<td>225</td>
</tr>
<tr>
<td>(14.0%)</td>
<td>(19.9%)</td>
<td></td>
<td>(49.6%)</td>
<td>(49.6%)</td>
<td></td>
<td>(16.9%)</td>
</tr>
</tbody>
</table>

Table II
Characterisation of Brucella isolates, from camels and cattle, to species and biovar levels

Isolates 1 to 3 were from camels and 4 to 6 from cattle.

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Reaction with monospecific antisera</th>
<th>Lysis with phages at RTD</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>M</td>
<td>Wb</td>
</tr>
</tbody>
</table>

R/TD: routine test dilution

Tb: Tbilise

TH: thionine at 20 µg/ml (1/50,000 w/v)

Wb: Weybridge
Discussion

The RBPT is widely used in Sudan for brucellosis screening for regulatory control and for export requirements. The RBPT is very sensitive and is suitable for screening herds for brucellosis, but it can give false positive results due to vaccination with B. abortus strain 19 vaccine or cross-reactions with other bacteria. The RBPT has been reported to have high sensitivity, therefore false negative responses are reported to occur less frequently than false positive responses (14). In an effort to evaluate the potential for false negative samples to occur with the RBPT test in camels, this study used the cELISA to re-evaluate RBPT negative samples. Based on serology alone, the cELISA data indicated that the RBPT may have missed 2.1% of seropositive camels. Given that the RBPT is used widely in Sudan, false negative results could have a negative impact on brucellosis control policies. The present data suggest that the cELISA may be useful as a second screening test for confirmation of RBPT results (14). The data also indicate that the mRBPT facilitates the reading of agglutination reactions (8) and is recommended for screening camel serum samples for the disease. Others (6) have reported that ELISAs to detect brucellosis are more sensitive than the RBPT, but have only marginally greater specificity than the RBPT or complement fixation test.

The data presented here suggest that the prevalence of brucellosis in camels in the Sudan is generally low, but may be increasing (13). Previous data on brucellosis in camels in the same localities in Sudan found 12.3% and 15.5% seroprevalence (7), whereas this study found the seroprevalence in this area to be 37.5%. The results presented here are similar to those of other studies that found seroprevalence rates for brucellosis in Sudanese camels to range from 1.8% to 43.9%. Thus, Abu Damir et al. (1) reported seroprevalence of 2%, 3.1% and 7.5% in 750 camels in central, western and eastern Sudan, respectively; Bitter (4) found the prevalence of brucellosis to be 26.5% in camels in eastern Sudan; Yagoub et al. (15) found 1.82% seroprevalence in 79 young camels, 6.95% in adult males and 13.77% in adult females of 1,502 camels in the same localities as studied here; Musa (11) reported 7.76% of 1,314 camels to be seropositive in Darfur, western Sudan; and Majid et al. (9) found seroprevalence of 13.9% and 43.9% in camels in different localities in Sudan.

Antibodies detected in suckling calves of 6 to 12 months of age could be attributed to passive transfer of maternal antibodies (3). Antibody titres in immature camel calves (one to three years) might be due to persistent Brucella infections. This has also been observed in cattle (11). The higher prevalence rates observed in females than in males, and in adults than in younger camels, in this study are consistent with other reports (2, 11, 15).

Epidemiological factors that contribute to the spread of brucellosis in camels in Sudan may include mixing of infected camels with healthy animals, mixed herding of different animal species, lack of brucellosis control measures and lack of knowledge of brucellosis by nomads. Human routes of exposure to infection most likely include: consumption of raw milk, occupational exposure, and lack of adequate protective measures in abattoir workers. Use of measures such as vaccination and test and slaughter to control brucellosis in Sudan is urgently needed.

Isolation of the same biovar of B. abortus and the atypical variant from cattle and camels indicates that the current husbandry methods may disseminate brucellosis between cattle and other species. These data and those of others (2) suggest that the causative agent of brucellosis in Sudan is B. abortus.

Fig. 3
Congested and haemorrhagic mesenteric lymph nodes and testes of a camel, from which Brucella organisms were isolated.
La brucellose chez les chameaux, les bovins et l’homme : association et évaluation de plusieurs tests sérologiques pour le diagnostic de la maladie dans certaines localités nomadiques du Soudan

M.M. Omer, M.T. Musa, M.R. Bakhiet & L. Perrett

Résumé
Lors d’une étude sur la brucellose effectuée au Soudan oriental, des échantillons de lait et de sérum prélevés sur 2 225 chameaux et des prélèvements de sérum collectés sur 20 chameleurs nomades et 33 travailleurs des abattoirs ont été analysés. La présence de Brucella a été recherchée par isolement à partir de tissus de ganglions lymphatiques, des testicules et de la mamelle de chameaux positifs, ainsi que du liquide obtenu par ponction des poches d’hygroma chez des vaches atteintes. Les échantillons sériques ont été soumis à l’épreuve au rose Bengale sur plaque (RBPT), à l’épreuve RBPT modifiée, au test de séro-agglutination (TSR) et à l’épreuve immuno-enzymatique (ELISA) de compétition ; les échantillons de lait ont été soumis à l’épreuve de l’anneau (ring test).

La prévalence sérologique globale était de 37,5 %. La prévalence sérologique était de 28,2 % chez les mâles et de 40,1 % chez les femelles. Douze chameliers nomades (60 %) et trois travailleurs des abattoirs (9 %) possédaient également des anticorps. Brucella abortus biovar 6 a été isolée de deux chameaux et de trois vaches. Deux isolats, obtenus l’un chez un chameau et l’autre chez une vache se sont avérés atypiques. Les résultats bactériologiques permettent de penser que l’infection a été transmise aux chameaux par les bovins, qui sont les hôtes primaires de B. abortus. L’épreuve RBPT modifiée a été jugée utile en tant qu’épreuve sérologique de dépistage chez les chameaux, mais l’ELISA de compétition s’est avérée plus sensible (2,1 % de résultats positifs détectés en plus). Les concentrations d’anticorps mises en évidence par TSR étaient comprises entre <13 et 3 282 UI/ml.

Mots-clés
Bovin – Brucella abortus biovar 6 – Brucellose – Chameau – Homme – Soudan.

Brucelosis en camellos, bovinos y humanos: asociaciones y evaluación de pruebas serológicas utilizadas para diagnosticar la enfermedad en ciertas localidades de nómadas de Sudán

M.M. Omer, M.T. Musa, M.R. Bakhiet & L. Perrett

Resumen
Los autores describen un estudio de la brucelosis en el Sudán oriental, en el cual se analizaron muestras de suero y leche de 2 225 camellos y muestras séricas de 20 camelleros nómadas y 33 operarios de matadero. Se trataba de aislar brucelas a partir de tejidos de ganglio linfático, testículo y ubre de camellos.
positivos y del líquido aspirado de higromas de tres vacas afectadas. Las muestras séricas fueron sometidas a las técnicas siguientes: aglutinación en placa de rosa de bengala (RB); prueba de rosa de bengala modificada (RBm); prueba de seroaglutinación (SA); y ensayo inmunoenzimático de competición (ELISAc). Asimismo, se someteron las muestras de leche a la prueba del anillo lácteo.

La seroprevalencia global resultó de un 37,5%. La seroprevalencia fue del 28,2% en los machos y del 40,1% en las hembras. Doce (60%) nómadas y tres (9%) técnicos de matadero mostraron títulos positivos de anticuerpos. En dos camellos y tres vacas se aisló el biovar 6 de *Brucella abortus*. En dos de esas muestras, una de cada especie, la cepa aislada era atípica. De las observaciones bacteriológicas se dedujo que las vacas eran el huésped primario de *B. abortus* y que ellas habían contagiado a los camellos. Se comprobó que la técnica de RBm resultaba útil para las pruebas sistemáticas de detección de brucelosis en el suero de los camellos, si bien con el ELISA de competición se detectó un número superior de positivos (un 2,1% más). Las concentraciones de anticuerpos observadas con la técnica de SA oscilaban entre < 13 y 3.282 UI/ml.

**Palabras clave**


References


