Rates of seroconversion in the progeny of *Brucella abortus* seropositive and seronegative cattle and buffalo

S. AKHTAR and M.A. MIRZA *

**Summary:** The authors estimated the rates of development of serum antibodies against *Brucella abortus*, between January 1987 and December 1990, in female Jersey cattle and buffalo weaned from seropositive and seronegative dams, and used logistic regression analysis to examine the epidemiological relationship of these rates with the serum antibody status of dams. The offspring from both seropositive and seronegative dams were reared together in calf pens, while separate pens were used for bovine and buffalo calves. Each of the bovine calves was manually fed two litres of bulked milk (pooled from seronegative and seropositive cows) twice a day, in the morning and evening. The buffalo calves, however, were allowed to suckle their respective dams before and after manual milking of each buffalo, in the morning and evening. Bovine calves and buffalo calves were weaned at approximately six months of age and moved to sheds for young livestock. At maturity, the female offspring were artificially inseminated, and pregnancy was subsequently diagnosed by rectal palpation.

The rate of seroconversion in the progeny of seropositive Jersey cattle was 26.4% (14 of 53 animals) compared to 14.3% (11 of 77 animals) in the progeny of seronegative cattle; this difference was non-significant (P = 0.1342). In buffalo, however, the rate of seroconversion in the progeny of seropositive dams (43.3% = 13/30) was significantly higher (P = 0.0002) than in the progeny of seronegative buffalo (8.8% = 6/68). The logistic regression analysis revealed that the progeny of seropositive buffalo were approximately 6.2 times more likely to have developed serum antibodies by the time of first calving than the progeny of seronegative buffalo (adjusted odds ratio [OR] = 6.2; 95% confidence interval [CI] = 1.5, 36.4). This relationship was non-significant, however, for the progeny of seropositive cattle (adjusted OR = 2.1; 95% CI = 0.6, 7.4). The implications of these results and potential sources of bias are discussed.

**KEYWORDS:** Brucellosis - Buffalo - Epidemiology - Jersey cattle - Logistic model - Pakistan - Spread.

**INTRODUCTION**

The bovine fetus may become infected with *Brucella abortus* in utero or during parturition (12) and/or through ingestion of colostrum or milk from an infected dam (9, 13). Subsequent persistent infection may be prevented in such calves, if they are removed from
the source of infection after the first few nursing periods (7). Some researchers believed that calves born to *B. abortus*-infected cows or fed on infected milk eventually become free from the infection and, therefore, cease to present a risk to other cattle (11, 17). Another study (18), however, suggested that the female progeny of infected cows can harbour the infection until their first pregnancy, which might terminate in abortion or normal calving and excretion of *B. abortus*. Infection acquired *in utero*, at birth or in early life may therefore remain dormant for months or even years, before resulting in seropositivity or disease in an unknown proportion of cases (15).

The frequency of seroconversion in the progeny of seropositive dams is unknown; it may range between 2.5% and 9% (14, 19), although the studies quoted were concerned with the problem of persistence of infection in progeny without serological response prior to abortion or infectious calving. Furthermore, the prevalence of infection in the progeny in these studies was not determined in an endemically-infected population, and no controls were included. A substantial proportion of calves which were separated from infected dams before the age of six months and raised in a brucellosis-free environment were found to be free from the disease (16). Thus the risk of seroconversion in progeny in the infected herds varies, depending on the calf-rearing practices and management of the herd.

The objectives of this study were to investigate the relative rates of seroconversion of female bovine and buffalo calves weaned from *B. abortus* seropositive and seronegative dams, and to examine the epidemiological relationship of these rates with the *Brucella* antibody status of the dam, after adjusting for the age of the progeny at first calving. This study provides an opportunity to quantify the risk of development of *Brucella* antibodies in serum by progeny born to seropositive dams, and the risk posed by environmental exposure, namely feeding of bulked milk (pooled from *B. abortus* seropositive and seronegative cows) or contact with bedding contaminated by infected companions during calfhood.

**MATERIALS AND METHODS**

**Herds**

The animals used in this study came from a herd of Jersey cattle and a herd of buffalo, both maintained at a livestock research station which had a history of *B. abortus* infection. Over a period of four years, between January 1987 and December 1990, both herds were serologically tested approximately every six months for evidence of infection with *B. abortus*. When serological evidence of brucellosis was detected, the animals were segregated from animals testing negative, and they were subsequently housed, managed and fed separately. The dams of both species were considered seronegative for *Brucella* if they tested serologically negative throughout the study period. Cattle and buffalo from both groups were culled for slaughter if they were of advanced age and had poor productive and/or reproductive efficiency.

**Management of the individual heifers studied**

The female progeny of *Brucella* seropositive and seronegative cattle and buffalo were ear-tagged at birth and identified with respect to their dams. The female progeny of both seropositive and seronegative dams were reared together in calf pens, while separate pens were used for bovine and buffalo calves. The bovine calves were each fed
two litres of bulked milk (pooled from seronegative and seropositive cows) manually in the morning and evening. The buffalo calves, however, were allowed to suckle their respective dams before and after manual milking of each buffalo in the morning and evening. Bovine and buffalo calves were weaned at approximately six months of age and moved to sheds for young livestock. At maturity, the female progeny were artificially inseminated, and pregnancy was subsequently diagnosed by rectal palpation. Pregnant and non-pregnant heifers were managed and fed separately, and were segregated into calving pens towards the end of pregnancy.

Sample collection

Each time the cattle and buffalo were serologically tested, blood samples were also collected from the female offspring chosen for the study. The first test was performed in January 1987, and subsequently the dams and female offspring were tested approximately every six months until December 1990. Blood samples were collected from female progeny of cattle and buffalo immediately after their parturition. A final blood sample was collected from female progeny of both cattle and buffalo one month after their calving.

Laboratory tests

Serum samples were tested for the presence of antibodies against \textit{B. abortus} antigen (obtained from the National Veterinary Services Laboratories [NVSL] in Ames, Iowa, United States of America) using the Rose Bengal plate test and the standard tube agglutination test. The animals were considered seropositive if positive reactions were obtained in both tests (at a 1:80 dilution in the tube agglutination test, as recommended by manufacturers [NVSL] and reported by others: 4, 10). Animals yielding positive results by the Rose Bengal test, but tube agglutination titres of less than 1:80, were considered suspect and were re-tested three to four weeks later. Few animals fell into this category, however, and all proved to be \textit{Brucella} positive in subsequent testing. Progeny were classified as \textit{Brucella} seropositive if seroconversion was observed at least until final testing, one month post-parturition.

Statistical analysis

The mean differences in the age at first calving of seronegative and seropositive progeny of both cattle and buffalo were compared using the student's \(t\) test. For each species, the proportion of progeny which seroconverted for each category of serum antibody status in dams was computed. The homogeneity of these proportions was tested using chi-square (\(\chi^2\)) analysis (5). Multivariate logistic regression analysis was performed, to measure the effect of the antibody status of the dam on the risk of seroconversion in progeny, after adjusting for the confounding effects of age at first calving (as age is an important factor in susceptibility to \textit{B. abortus} infection) (1). Separate logistic models were employed for cattle and buffalo. Logistic coefficients and their standard errors were used to compute adjusted odd ratios (OR) and 95\% confidence intervals (CI) (8).

RESULTS

The mean age at first calving (± standard deviation) did not differ significantly between seropositive and seronegative progeny of cattle (\(P = 0.829\)) or buffalo (\(P = 0.721\)) (Table I). This variable was included in the final logistic models, however, to adjust for its residual confounding effect.
TABLE I

Descriptive statistics on 'age at first calving' of Brucella seropositive and Brucella seronegative Jersey cattle and buffalo, January 1987 to December 1990

<table>
<thead>
<tr>
<th>Species</th>
<th>Serological status</th>
<th>No. of animals</th>
<th>Mean age (months)</th>
<th>SD</th>
<th>Student's t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Negative</td>
<td>105</td>
<td>21.9</td>
<td>1.1</td>
<td>1.80</td>
<td>0.829</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>25</td>
<td>24.9</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo</td>
<td>Negative</td>
<td>79</td>
<td>38.0</td>
<td>2.7</td>
<td>0.36</td>
<td>0.721</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>19</td>
<td>39.0</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation

Table II shows the total number of female progeny weaned from B. abortus seropositive and seronegative cattle and buffalo, and rates of seroconversion in the progeny. The rate of seroconversion in the progeny of seropositive Jersey cattle was 26.4%, compared to 14.3% in the progeny of seronegative cattle, but this difference was not statistically significant ($P = 0.1342$). The rate of seroconversion in the progeny of seropositive buffalo (43.3%), however, was significantly ($P = 0.0002$) higher than in the progeny of seronegative buffalo (8.8%).

TABLE II

Statistical record of calving and serological status of progeny born to Brucella seronegative and Brucella seropositive cattle and buffalo, January 1987 to December 1990

<table>
<thead>
<tr>
<th>Species</th>
<th>Serological status of dams</th>
<th>Total no. of female progeny</th>
<th>No. of female progeny seroconverted (%)</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>Positive</td>
<td>53</td>
<td>14 (26.4)</td>
<td>2.2</td>
<td>0.1342</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>77</td>
<td>11 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo</td>
<td>Positive</td>
<td>30</td>
<td>13 (43.3)</td>
<td>13.7</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>68</td>
<td>6 (8.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III presents the results from logistic models of the relationship between B. abortus antibody status in dams and the rate of seroconversion of progeny, after accounting for the residual effect of the age of the progeny at first calving. The progeny of seropositive buffalo were 6.2 times more likely to possess serum antibodies around the time of first calving than the progeny of seronegative buffalo (adjusted OR = 6.2; 95% CI = 1.5, 36.4). For the progeny of seropositive cattle, however, this relationship was non-significant (adjusted OR = 2.1; 95% CI = 0.6, 7.4).

DISCUSSION

The Rose Bengal plate test and the standard tube agglutination test were used to test experimental animals for antibodies against B. abortus, as these tests have been widely employed in many successful national brucellosis eradication programmes (4, 10).

Certain precautions must be taken in the interpretation of the data obtained. The most important potential bias could have occurred in classifying the seronegative status
Multivariate logistic regression models of rate of seroconversion in the progeny of \textit{Brucella} seropositive and \textit{Brucella} seronegative cattle and buffalo

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Odds ratio *</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (for Jersey cattle)</td>
<td>DAMST</td>
<td>1.6</td>
<td>(0.5, 7.4)</td>
</tr>
<tr>
<td></td>
<td>AGEFC</td>
<td>0.9</td>
<td>(0.2, 1.3)</td>
</tr>
<tr>
<td>Model 2 (for buffalo)</td>
<td>DAMST</td>
<td>6.2</td>
<td>(1.5, 36.7)</td>
</tr>
<tr>
<td></td>
<td>AGEFC</td>
<td>0.6</td>
<td>(0.1, 1.8)</td>
</tr>
</tbody>
</table>

* associated with an increase of one standard deviation in value of risk factor

DAMST: dam serological status against \textit{Brucella abortus} (0 = negative; 1 = positive)
AGEFC: age at first calving (months) in progeny

of dams and their progeny, due to the specificity of the tests and the stage of \textit{B. abortus} infection: seronegative status of experimental animals in an infected herd does not necessarily mean that the animals are free from infection. However, the validity of assuming freedom from infection in animals which repeatedly gave negative serological results could be supported by the results of a report (1) that 31% permanent non-reactors were found in infected herds on repeated testing over a five-year period. Another possible source of bias is that female progeny of seropositive and seronegative dams were housed together until one or more tested \textit{Brucella} positive; the seropositive animals were then isolated. Therefore, there may have been horizontal transfer of infection. Partial adjustment was made for this possibility, however, by including appropriate control groups in logistic regression analysis.

A herd usually becomes diseased due to the introduction of \textit{Brucella}-infected animals, and infection subsequently spreads within the herd from aborted fetuses, placenta and genital discharges, which contaminate farm premises (2). Susceptibility of calves is relatively low, but they may acquire \textit{B. abortus} infection either \textit{in utero} (12) or by ingesting infected colostrum or milk (9, 13), which may be intermittently infected for several months after calving. Infected calves may then be responsible for the spread of infection to other calves in the pen through their excreta, and to other animals by contaminating the environment (11, 17).

Several studies have indicated that latent \textit{Brucella} infection can develop in some calves exposed to virulent \textit{B. abortus} either \textit{in utero} or during early calfhood (3, 6, 15). Affected females usually show no signs of disease until late gestation, or until they have calved or aborted. The frequency of such latent infection and subsequent potential seroconversion in progeny varies, depending on farm management practices. The present study provided an opportunity to quantify the risk of seroconversion in progeny associated with seropositivity in the dam. This risk is additional to the risk presented by feeding pooled milk (pooled from seronegative and seropositive cows) and by a potentially-contaminated environment.

The analysis of the data revealed a non-significant relationship in cattle between serological status of dams and the rate of seroconversion in progeny. Other studies (9, 15) have recorded 18-20% rates of seroconversion in the progeny of seropositive dams, which is similar to the rate (26.4%) recorded in the present study. The 14.3% rate of seroconversion in the progeny of seronegative cattle in this study was substantially
higher than the figure of 1.0% reported in an earlier study (6). A substantial but non-significant difference in rates of seroconversion in the two groups of offspring in the present study reflects the uniform, but poor, management of the calves born of seropositive and seronegative cattle. Therefore, calves from known infected or seropositive dams should be slaughtered, and all the calves should be fed on milk from Brucella-free cows, to prevent cross-infection.

The rate of seroconversion in the female progeny of B. abortus seropositive buffalo was significantly higher than in the female progeny of B. abortus seronegative buffalo. To the knowledge of the authors, this is the first study of relative rates of seroconversion in the progeny of B. abortus seropositive and seronegative buffalo, and therefore no published data is presented to contrast these findings. These observations can be explained, however, in terms of management practices used for buffalo. Limited calf-suckling is used for 'let-down' of milk in buffalo before milking, and calves are also allowed to suckle their respective dams after milking in both private and state-owned herds in Pakistan. Since calves suckled their respective B. abortus seropositive and seronegative dams, this resulted in a greater likelihood of infection being contracted by calves born to seropositive buffalo (through repeated exposure due to nursing) than by calves born to seronegative buffalo. The present study yielded information on managerial factors which possibly contributed to perpetuating Brucella infection in a state-owned herd.

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Résumé : Les auteurs ont étudié le développement des anticorps vis-à-vis de Brucella abortus dans les séums prélevés, entre janvier 1987 et décembre 1990, sur des génisses jersiaises et de jeunes bufflesses, sevrées par des mères possédant elles-mêmes (ou non) de tels anticorps. Ils ont ensuite effectué une analyse de régression logistique de la relation épidémiologique entre ces taux et le statut immunitaire des mères. Les descendants de mères possédant ou non des anticorps ont été élevés dans les mêmes enclos tandis que les veaux et bufflons ont été parqués séparément. On a alimenté les veaux manuellement en donnant à chacun, deux fois par jour, matin et soir, deux litres de lait (provenant aussi bien de vaches ne possédant pas d'anticorps que possédant des anticorps). Les bufflons pouvaient, quant à eux, téter leurs mères respectives avant et après la traite manuelle. Veaux et bufflons ont été sevrés à l'âge de six mois environ et placés dans des bâtiments pour jeunes animaux. A la puberté, les descendantes femelles ont été soumises à une insémination artificielle et le diagnostic de la gestation a été effectué par exploration rectale.

Le taux de séroconversion dans la descendance de vaches jersiaises possédant des anticorps était de 26,4 % (14 sur 53 animaux) contre 14,8 % (11 sur 77 animaux) pour la descendance de vaches ne possédant pas d'anticorps, soit une différence peu significative (P = 0,1342). Chez les buffles, en revanche, le taux de séroconversion était nettement supérieur (P = 0,0002) dans la descendance de mères possédant des anticorps (43,3 % = 13/30) par rapport à celle de mères n’en possédant pas (8,8 % = 6/68). L'analyse de régression logistique a révélé que les descendantes de bufflesses possédant des anticorps avaient 6,2 fois plus de chances de développer des anticorps dans le sérum au
moment du premier vêlage que celles de buffles sans anticorps (adjusted odds ratio : [OR] = 6,2 % ; intervalle de confiance [IC] de 95 % = 1,5-36,4). Ce rapport n'était cependant pas significatif pour la descendance de vaches possédant des anticorps (OR ajusté = 2,1 ; IC95 % = 0,6-7,4). Les conséquences de ces résultats et les sources potentielles d'erreur font l'objet de la discussion.

MOTS-CLÉS : Brucellose - Bufflesse - Epidémiologie - Modèle logistique - Pakistan - Propagation - Vaches jersiaises.

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Resumen: Entre enero de 1987 y diciembre de 1990, los autores estimaron las tasas de desarrollo de anticuerpos séricos contra Brucella abortus en hembras de raza Jersey y de búfalo destetadas tanto de madres seropositivas como seronegativas. Después se sirvieron del análisis de regresión logística para estudiar la relación epidemiológica que dichas tasas guardan con la condición de seropositividad o seronegatividad de la madre. Las crías de madres tanto seropositivas como seronegativas se criaron juntas en los mismos corrales, aunque se separaron en corrales distintos las crías Jersey y las crías de búfalo. Cada una de las crías bovinas era alimentada con dos litros de leche a granel (procedente tanto de vacas seronegativas como seropositivas), leche que le era administrada manualmente dos veces al día, por la mañana y por la tarde. A las crías de búfalo, en cambio, se les permitió lactar de sus respectivas madres antes y después del ordeño manual de la mañana y de tarde. Las crías bovinas y las de búfalo fueron destetadas aproximadamente a los seis meses de edad y trasladadas a cobertizos para ganado joven. Al llegar a la madurez las hembras fueron inseminadas artificialmente, y la subsiguiente gravidez se diagnosticó mediante palpación rectal.

La tasa de seroconversión en la progenie de vacas Jersey seropositivas fue de un 26,4% (14 de 53 animales). Para las crías de vacas seronegativas se observó una tasa del 14,3% (11 de 77 animales). Esta diferencia no era significativa (P = 0,1342). En el caso del búfalo, sin embargo, la tasa de seroconversión para las crías de madres seropositivas (43,3% = 13/30) resultó significativamente mayor que la registrada entre la progenie de madres seronegativas (8,8% = 6/68). El análisis de regresión logística reveló que la descendencia de madres búfalo seropositivas tenía una probabilidad de haber desarrollado anticuerpos séricos antes del primer parto aproximadamente 6,2 veces mayor que la progenie de búfalo seronegativas (coefficiente de probabilidad [OR] ajustado = 6,2; intervalo de confianza 95% [CI] = 1,5-36,4). Esta relación, en cambio, era no significativa en el caso de la progenie de vacas seropositivas (OR ajustado = 2,1; CI 95% = 0,6-7,4). Los autores discuten las implicaciones de estos resultados y las posibles fuentes de desviación.

REFERENCES


