SEROLOGICAL STUDIES ON BOVINE BESNOITIOSIS IN ISRAEL

M. GOLDMAN and E. PIPANO
Kimron Veterinary Institute, Bet Dagan, Israel

SUMMARY
Sera from 1,700 beef and dairy cattle in Israel were tested for besnoitiosis by indirect immunofluorescence. Over 90% of dairy animals were negative whereas about 50% of beef cattle were positive. Among beef bulls the percentage of positive reactors increased with age but in beef cows the pattern was less clear; 36% of young imported bulls negative upon arrival developed antibody titres after one season on the range. Cross-tests with Toxoplasma gondii antiserum and antigen indicated that the results of the survey for besnoitiosis were unlikely to have been influenced by Toxoplasma antibodies.

INTRODUCTION
Bovine infections with Besnoitia besnoiti have been recognised in Israel on the basis of clinical, histopathological and serological studies (Neuman and Noble, 1960; Neuman, 1972; Frank, Pipano and Rosenberg, 1977). The present report is an expansion of previous serological studies to include cattle drawn from more geographic areas and followed over a longer period of time. In addition we report results of cross-testing anti-Besnoitia sera with Toxoplasma gondii antigen.

MATERIALS AND METHODS
Cattle were tested from 19 herds located in the northern hilly part of Israel and 28 from the valleys and coastal plain. Seven of the herds consisted of dairy cows (Israeli Friesian), two of dairy bulls from artificial insemination centres and the rest of bulls and cows in beef herds (Charolais, Siementhal, Gelfi, Brahman or local crossbred variants). A total of 1,877 sera were tested from 1,700 cattle.

Serum was tested by indirect immunofluorescence with slide antigen prepared from B. besnoiti grown in BHK or Vero cells. Heavily infected monolayer cells were brought into suspension in the usual manner with versene or trypsin, centrifuged and the pellet was resuspended in phosphate-buffered saline containing 1% formalin. After 1 h the suspension was recentrifuged, the pellet washed and resuspended in saline. Drops of suspension were allowed to dry on Teflon-sprayed slides in an eight-well per slide configuration (Goldman, 1968) and the slides were stored at -20°C. For use slides were dipped in acetone for 5 min and dried. Smears were exposed for 1 h to four-fold dilutions of serum from 1:16 to final titre and then to fluorescent anti-bovine conjugate (1:100) for 1 h. The positive control consisted of serum from a bull heavily infected in the perigenital region with typical Besnoitia cysts. Toxoplasma gondii antigen used in the cross-reaction tests was prepared from peritoneal exudate of gerbils infected with the RH strain of T. gondii. The parasites had been stored frozen for about 10 years before being retrieved by inoculation into the gerbils. The Toxoplasma slide antigen was prepared in the same manner as the Besnoitia antigen.

Antiparasite sera conjugated with fluorescein isothiocyanate were used for direct immunofluorescent staining of T. gondii and B. besnoiti. Conjugated anti-T. gondii
antiserum was prepared from pooled sheep sera that showed high titres in indirect fluorescent antibody tests; conjugated anti- \textit{B. besnoiti} antiserum was prepared from the same serum used as positive control in the direct test for \textit{Besnoitia}. In both cases serum proteins precipitated by 50\% saturated ammonium sulphate were labelled with fluorescein isothiocyanate and cleared of unreacted dye by passage through Sephadex G-25 (Goldman, 1968).

**RESULTS**

**Field cattle**

No consistent pattern of results was associated with any specific geographic location or type of terrain in which the herds were located. For this reason the results from all of the herds are grouped together.

Table I shows the distribution of titres found in beef and dairy bulls and cows. Dairy animals showed the lowest percentage of positives at titres of 1:64 or higher, 8\% and 1\% for cows and bulls respectively, whereas the comparable figure for beef cows was 40\% and for beef bulls 57\%. Furthermore while the highest titre found among dairy animals was 1:64, the beef herds yielded titres of 1:1,024 or higher in 5\% of the cows and 13\% of the bulls.

Figure 1 shows the percentage distribution of titres for each sex separately in 332 bulls and 356 cows drawn from the same six herds. Although the differences between the sexes are not great there appears to be a consistent pattern of a greater incidence of higher titres in bulls than in cows.

Figures 2 and 3 show that the percentage distribution of titres according to age differed considerably in the two sexes. Bulls showed a clear shift with increasing age in the direction of more positives and higher titres, going from about 10\% positives in the under one year group to 75\% positives in the four or more years group. Among cows the highest percentage of positives occurred in the two year old group, 45\%, dropping to 25\% in the four or more years old group. In addition in cows there was no shift towards higher titres with increasing age.

Thirty-nine yearling bulls imported in November to December 1977 were tested upon arrival and again in July or September 1978 after having been distributed

<table>
<thead>
<tr>
<th>Type of herd</th>
<th>Number of sera (%)</th>
<th>Neg. or 1:16</th>
<th>1:64</th>
<th>1:256</th>
<th>1:1,024 or greater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef bulls</td>
<td>1,133 (100)</td>
<td>486</td>
<td>196</td>
<td>300</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef cows</td>
<td>367 (100)</td>
<td>219</td>
<td>73</td>
<td>57</td>
<td>18</td>
</tr>
<tr>
<td>Dairy bulls</td>
<td>93 (100)</td>
<td>92</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dairy cows</td>
<td>284 (100)</td>
<td>260</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,877</td>
<td>1,877</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Titres of 1:16 have been grouped with the negatives as a realistic interpretation of the test. The number of very recent and perhaps very old infections possibly missed as a result was considered insignificant judging by results of experimental infections to be reported elsewhere.