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# COMPARATIVE STUDIES OF *BABESIA* SPP. FROM WHITE-TAILED AND SIKA DEER

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ABSTRACT: *Babesia odocoilei* from white-tailed deer (*Odocoileus virginianus*) in Texas (USA) and *B. capreoli* isolated from sika deer (*Cervus nippon*) in Ireland were compared morphologically and antigenically. *Babesia odocoilei* and *B. capreoli* paired pyriforms resembled each other closely when in sika deer, but *B. odocoilei* pyriforms in white-tailed deer were slightly different. *Babesia odocoilei* in white-tailed deer also differed from *B. odocoilei* and *B. capreoli* in sika deer in the frequency of its developmental forms. Indirect immunofluorescence antibody test titres showed that there was some antigen cross-reactivity, but not as much as between *B. capreoli* and the bovine parasite, *B. divergens*. The *Babesia* spp. from deer that we studied appear to be distinct but related species. The low infectivity of *B. odocoilei* for a splenectomised sika deer suggests that sika deer in North America are probably not very susceptible to this parasite in the wild.

*Key words:* Sika deer, *Cervus nippon*, white-tailed deer, *Odocoileus virginianus*, *Babesia odocoilei*, *Babesia capreoli*, morphology, antigenicity.

## INTRODUCTION

A hemoparasite of the white-tailed deer (*Odocoileus virginianus*) found in Texas and named as *Babesia odocoilei* by Emerson and Wright (1970) was observed to be morphologically similar to the cattle parasite *Babesia divergens* (Waldrup et al., 1990). In Scotland *Babesia capreoli* from red deer (*Cervus elaphus*) was also found to resemble *B. divergens* in many respects (Adam et al., 1976). However, neither *B. odocoilei* nor *B. capreoli* were transmissible to cattle in these studies.

It is possible that *B. capreoli* and *B. odocoilei* are closely related or even identical species. Circumstantial evidence suggests that the tick *Ixodes ricinus* is the vector for *B. capreoli* (Adam et al., 1976) and another member of the *Ixodes ricinus* species complex, *I. scapularis*, transmits *B. odocoilei* (K.A. Waldrup, unpubl.). *Babesia capreoli* also has been found in sika deer in Ireland (Gray et al., 1990). Red and sika deer (*Cervus nippon*) are very closely related with some authorities considering them to be subspecies (Harrington, 1979), but white-tailed deer belong to a different genus. In this study morphological and antigenic comparisons were made between *B. odocoilei* in sika and white-tailed deer and *B. capreoli* in sika deer.

## MATERIALS AND METHODS

*Babesia odocoilei*-infected blood was obtained from a 7-mo-old male white-tailed deer that had been transfused with 200 ml of pooled, citrated blood taken from wild white-tailed deer in the Welder Wildlife Refuge, on the central Gulf Coast of Texas (97°25'W, 27°06'N). The deer was given 2 mg of dexamethasone (Azium, Schering Corporation, Kenilworth, New Jersey 07033, USA) daily for 5 days before infection. Erythrocytic parasites were seen on Giemsa stained thin blood smears on day 7 post-inoculation. On day 10 post-inoculation fresh blood smears were made and about 20 ml blood was taken by jugular puncture into citrate solution when the parasitemia was about 11%. The blood was kept at 4 C while being flown from Texas A&M University (College Station, Texas 77843, USA) to the Veterinary Research Laboratories (VRL, Abbotstown; Abbotstown, Dublin, Ireland). The blood was inoculated intravenously into a splenectomised sika deer 2 days after collection. *Babesia capreoli* from sika deer was iso-

lated in splenectomised deer at VRL, Abbotstown by inoculation of pooled whole blood from sika deer culled at Luggala Estate, County Wicklow, Ireland (53°08'N, 6°16'W).

Four sika deer were obtained when 4-mo-old from the Forest and Wildlife Service at Avondale, County Wicklow, Ireland. They were transported to VRL, Abbotstown and splenectomised 1 wk later. They were maintained together in a loose box and were infected two weeks after splenectomy. The sika deer, which had been reared under tick-free conditions, were sero-negative for *B. capreoli* (indirect immunofluorescence test) before any infections were conducted.

Sika deer one (SD1) was inoculated intravenously with 10 ml blood containing an estimated $4.5 \times 10^9$ erythrocytes infected with *B. odocoilei*. This animal was challenged 31 days later with erythrocytes infected with *B. capreoli* obtained from sika deer three (SD3).

Sika deer two (SD2) was infected with *B. capreoli* by the intravenous inoculation of 15 ml pooled blood collected in EDTA from three sika deer culled at Luggala, County Wicklow 24 hr earlier. The culled animals showed rare suspected babesias in thin blood smears. Dexamethasone (Dexafort, Intervet Ltd., Waterford, County Waterford, Ireland) was given to SD2 as a 3 ml intramuscular injection at the time of infection. On day 11 after infection, when the parasitemia had reached 6.5%, 2 ml of blood from this animal, containing approximately $8.0 \times 10^8$ infected erythrocytes, were injected intravenously into SD3.

Sika deer three (SD3) was bled on day 8 after infection, when the parasitemia had fallen to less than 0.1%, and 10 ml blood containing approximately $3.5 \times 10^7$ infected erythrocytes inoculated intravenously into SD1.

Sika deer four (SD4) was used in unsuccessful attempts to isolate *B. capreoli* from seropositive red deer blood obtained from the Moredun Research Institute, Edinburgh, UK and then from more culled sika deer blood from Luggala, County Wicklow. The sequence of these infections is illustrated in Figure 1.

Morphometric analysis was carried out on the parasites in Giemsa stained blood smears. It was not possible to obtain smears with comparable parasitemias from all animals. However, sika deer blood containing *B. odocoilei* and *B. capreoli* at low parasitemias was obtained from animals that recovered spontaneously. A smear of *B. capreoli* at a high parasitemia from SD1, which did not recover, was included for comparison with *B. odocoilei* in white-tailed deer.

One hundred parasites from each smear were classified and 25 round forms and 10 singlets of paired pyriforms were measured by means of

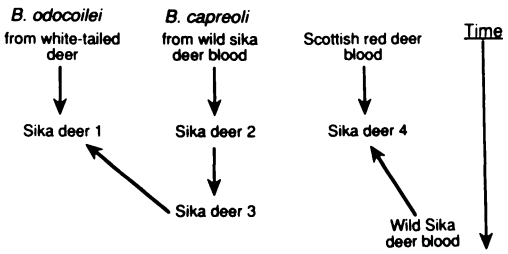


FIGURE 1. Sequence of infections of the sika deer with *B. odocoilei* and *B. capreoli*.

a camera lucida mounted on a Leitz Dialux microscope. The parasite dimension data were subjected to an analysis of variance and Duncan's multiple range test and the classification data to the Kolmogorov-Smirnov test (Siegel, 1956). Serological cross reactions between the two deer parasites as well as the bovine parasite, *B. divergens*, were analysed with the indirect immunofluorescence antibody test using a Leitz Dialux incident blue light microscope and a ×50 water objective. *Babesia odocoilei* antigen was prepared from culture material, *B. capreoli* antigen from infected blood from SD1 and *B. divergens* antigen from gerbils (*Meriones unguiculatus*). Following 3 × 5 min washes in phosphate buffered saline (pH 7.2) thick smears were prepared on glass microscope slides, air dried, covered with adhesive tape and frozen at −20 C. When used, wells were marked with a diamond pencil, slides fixed in ice-cold acetone for 10 min, acrylic circles placed around the wells and the slides washed for 15 min in phosphate buffered saline (PBS). The test sera were dispensed on the wells and incubated at 37 C for 1 hr in a humidifed incubator. After further washing in PBS, anti-bovine FITC conjugate (Sigma Chemicals Company Ltd., Fancy Road, Poole, Dorset, BH17 7NH, England) at 1:80 in PBS was dispensed and the slides incubated for another hour before thorough washing in PBS, mounting in buffered glycerol (pH 7.2) and examination. Anti-bovine rather than anti-deer FITC conjugate was used because of its commercial availability. Since the relative reactivity of different antigens to various sera was being measured the absolute sensitivity of the test was of secondary importance.

## RESULTS

Clinical reactions of sika deer to the two *Babesia* spp. are summarised in Table 1. It can be seen that only a transient infection with *B. odocoilei* was established with parasitemias never exceeding 0.1%. However, some changes in blood values were

TABLE 1. Reactions of sika deer to infections with *Babesia odocoilei* and *B. capreoli*.

| Deer number | Infection source | Pre-patent period | Highest % parasitemia | Patency period | Highest temperature elevation above initial value | Percentage PCV depression | Percentage RBC depression | Percentage Hb depression |
|---|---|---|---|---|---|---|---|---|
| 1 | White-tailed deer (*B. odocoilei*) | 4[a] | 0.1 | 5[a] | 0.3[b] | 23.7 | 19.3 | 18.7 |
| 2 | Sika deer culls (*B. capreoli*) | 8 | 19.8 | 8 | 2.1 | 82.3 | 84.9 | 85.2 |
| 3 | SD2 (*B. capreoli*) | 1 | 4.8 | 8 | 1.2 | 67.0 | 69.4 | 70.8 |
| 4 | Scottish red deer and sika culls | | No patency | | ND[c] | ND | ND | ND |

[a] Days.
[b] Centigrade.
[c] Not done.

evident. In contrast inoculation of carrier blood from wild sika deer into SD2 resulted in a severe infection with *B. capreoli* and, although parasitemias declined from day 10 and the animal was treated with diminazene aceturate (Berenil, Hoechst Ltd.) on day 14, it died on day 17. The severity of the infection was probably due to the treatment with dexamethasone since transfer of infected whole blood from this animal to SD3 did not result in acute babesiosis. However, the parasite became well established in SD3 and caused considerable changes in temperature and blood values before spontaneous recovery occurred. Whole blood from SD3 was transferred to SD1, which had been previously infected with *B. odocoilei*, but this infection did not become patent even though at least $3.5 \times 10^7$ infected erythrocytes were contained in the inoculum.

Many developing forms of *B. odocoilei* and *B. capreoli* in stained blood smears from sika deer were remarkably similar to each other, but the predominant paired forms of these parasites in sika and of *B. odocoilei* in white-tailed deer were noticeably different (Fig. 2).

Comparison of the dimensions of the parasites showed that *B. odocoilei* round forms were significantly smaller than when in white-tailed deer and also than those of *B. capreoli* in sika deer. No significant differences in the length of the paired pyriforms of the two babesia species were found, but the widths of *B. capreoli* paired pyriforms in sika deer were significantly greater than those of *B. odocoilei* in either deer species. These differences were also evident when the length : width ratios, a measure of the general shape of the pyriform, were compared (Table 2).

Most individuals of the two *Babesia* spp. occupied a peripheral intra-erythrocytic position in both deer species, but this was least marked for *B. odocoilei* in white-tailed deer. An analysis of the frequency distribution of developmental forms using the Kolmogorov-Smirnov test showed that there were statistically significant differences in four of the comparisons (*B. capreoli*/SD 0.1% *P* v *B. odocoilei*/W-T and *B. odocoilei*/SD, $\chi^2 = 36.98$; $P < 0.001$ and $\chi^2 = 6.48$; $P < 0.05$ respectively and *B. odocoilei*/SD v *B. odocoilei*/W-T, $\chi^2 = 23.12$; $P < 0.001$). The exception was where the level of parasitemia of *B. capreoli* in sika deer was the variable analysed ($\chi^2 = 0.72$; $P > 0.1$) (Table 3). The differences are due to differences in the frequency of occurrence of double pyriforms. When double pyriforms were combined with double round forms for data analysis the frequency distribution difference between

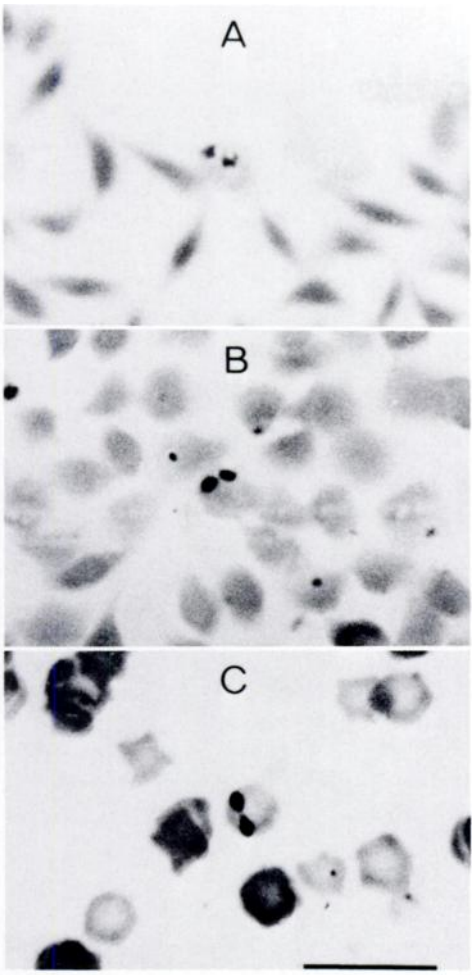


FIGURE 2. Paired pyriforms of *B. capreoli* in sika deer (A), *B. odocoilei* in sika deer (B) and *B. odocoilei* in white-tailed deer (C). (Bar = 10 μm.)

TABLE 3. Relative numbers (%) of developmental forms of *Babesia odocoilei* in white-tailed (W-T) and sika deer (SD) and *Babesia capreoli* in sika deer.

| | *B. capreoli* in SD | *B. capreoli* in SD | *B. odocoilei* in W-T | *B. odocoilei* in SD |
|---|---|---|---|---|
| % Parasitaemia | 19.8 | 0.1 | 9.5 | 0.1 |
| Single pyriform | 11 | 9 | 24 | 10 |
| Double pyriform | 5 | 5 | 35 | 15 |
| Single round | 40 | 47 | 26 | 54 |
| Double round | 20 | 14 | 2 | 3 |
| Oval/Elliptic | 17 | 21 | 8 | 14 |
| Elongate | 7 | 4 | 5 | 4 |
| Pyknotic or amoeboid | 8.2 | 13.0 | 16.7 | 63.0 |

*B. odocoilei*/WT and *B. capreoli*/SD was reduced but remained significant ($\chi^2$ = 20.48, $P < 0.001$). However, that between *B. odocoilei*/SD and *B. capreoli*/SD became non-significant ($\chi^2 = 0.72$, $P > 0.1$) when double pyriforms and double round forms were analysed together. Degenerating forms (pyknotic or amoeboid) were much more in evidence in *B. odocoilei* in sika deer than *B. odocoilei* in white-tailed deer or *B. capreoli* in sika deer.

The IFA test showed that although cross reactions occurred between *B. odocoilei*, *B. capreoli* and *B. divergens*, the least cross reactivity was evident between *B. odocoilei* and the other babesias. It was not possible to distinguish *B. divergens* and *B. capreoli* with this test. The tests were run at least three times for each antigen and showed good repeatability.

TABLE 2. Mean dimensions (±SE) expressed in μm and intraerythrocytic location of *Babesia capreoli* in sika deer (SD) and *B. odocoilei* in sika deer and white-tailed deer (W-T).

| | *B. capreoli* in SD | *B. capreoli* in SD | *B. odocoilei* in W-T | *B. odocoilei* in SD |
|---|---|---|---|---|
| Parasitaemia | 19.8 | 0.3 | 9.5 | 0.1 |
| Round | | | | |
| Diameter | 1.49 ± 0.03 | 1.46 ± 0.06 | 1.53 ± 0.04 | 1.20 ± 0.07 |
| Double Pyriform | | | | |
| Length | 1.48 ± 0.05 | 1.44 ± 0.046 | 1.52 ± 0.07 | 1.43 ± 0.09 |
| Width | 0.94 ± 0.04 | 0.93 ± 0.04 | 0.83 ± 0.04 | 0.85 ± 0.04 |
| Length : width ratio | 1.58 ± 0.07 | 1.57 ± 0.10 | 1.88 ± 0.12 | 1.71 ± 0.13 |
| Peripheral location (%) | 78 | 75 | 61 | 70 |

Means underscored by the same line are not significantly different at $P = 0.05$ (Duncan's Multiple Range Test).

## DISCUSSION

The results presented here suggest that *B. odocoilei* is related to but not identical with *B. capreoli*. *B. odocoilei* bore a marked resemblance to *B. capreoli* when in sika deer, the only differences being in the smaller round forms, which may be due to the apparent unsuitability of the sika deer as a host, and the slightly more rounded nature of the paired pyriforms of *B. capreoli*, which was evident from width measurements, the length : width ratios and the frequency classifications. The unsuitability of the sika deer as host for *B. odocoilei* was indicated by the low and transient parasitemia seen in SD1 and also by the large proportion of degenerating parasites observed in this animal.

It is apparent that both the morphology and the frequency of the various developmental forms can be influenced to some extent by the particular susceptibility of the individual hosts sampled, so that caution should be exercised when using these as taxonomic criteria. However, when considered in conjunction with other data, as here, the appearance of parasites in blood smears can make an important contribution to the assessment of their relative status.

Serological cross reactivity between *B. odocoilei* and *B. capreoli* was observed but this was at least two dilutions lower than in the homologous combinations, whereas *B. capreoli* gave the same titres with *B. divergens* in heterologous as in homologous combinations. *Babesia divergens* is readily distinguished from other bovine babesias with the IFA test (Gray and De Vos, 1981) and these results seem to indicate that *B. odocoilei*, *B. capreoli* and *B. divergens* are closely related to each other, with *B. odocoilei* less so than the latter two. The failure of *B. capreoli* to become established in SD1, which had previously been infected with *B. odocoilei*, may have been due to immunological cross-reactivity between the two species.

It is possible that the low infectivity of the *B. odocoilei* inoculum used here was due to adverse conditions experienced in the 48 hr transit period from Texas, but parasites appeared to be in good condition at infection and transferred successfully to sika erythrocytes in short term in vitro cultures using the methods of Holman et al. (1988). Sika deer are probably exposed to *B. odocoilei* in North America but no infections have been recorded and it is likely that sika deer are not good hosts for this parasite.

It should also be noted that sika deer, which are of Far Eastern origin, are probably not the normal host of the *B. capreoli* isolated in this study. High susceptibility was evident only when one of the splenectomised animals was treated with dexamethasone and recent survey evidence suggests that *B. capreoli* infection rates in Ireland are higher in red than in sika deer though both are exposed to ticks in the wild (Gray et al., 1990).

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## LITERATURE CITED

ADAM, K. M. G., D. A. BLEWETT, D. W. BROCKLESBY, AND G. A. M. SHARMAN. 1976. The isolation and characterisation of a babesia from red deer (*Cervus elaphus*). Parasitology 73: 1–11.

EMERSON, H. R., AND W. T. WRIGHT. 1970. Correction. Journal of Wildlife Diseases 6: 519.

GRAY, J. S., AND A. J. DE VOS. 1981. Studies on a bovine babesia transmitted by *Hyalomma marginatum rufipes* Koch 1844. Onderstepoort Journal of Veterinary Research 48: 215–223.

———, T. M. MURPHY, S. M. TAYLOR, D. A. BLEWETT, AND R. HARRINGTON. 1990. Comparative morphological and cross transmission studies with bovine and deer babesias in Ireland. Preventive Veterinary Medicine 9: In press.

HARRINGTON, R. 1979. Some aspects of the biology and taxonomy of the deer of the Co. Wicklow region, Ireland. Ph.D. Thesis. National University of Ireland, Dublin, Ireland, 149 pp.

HOLMAN, P. J., K. A. WALDRUP, AND G. G. WAGNER.

1988. In vitro cultivation of a babesia isolated from a white-tailed deer (*Odocoileus virginianus*). The Journal of Parasitology 74: 111–115.

SIEGEL, S. 1956. Non-parametric statistics for the behavioural sciences. McGraw Hill Ltd. Kogakusha, Tokyo, Japan, 312 pp.

WALDRUP, K. A., J. S. GRAY, AND G. G. WAGNER. 1990. Comparisons of *Babesia divergens* and *Babesia odocoilei*. Veterinary Parasitology 17: In press.

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