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# Culture Isolation and Partial Characterization of a *Babesia* sp. from a North American Elk (*Cervus elaphus*)

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ABSTRACT: Three North American yearling elk (*Cervus elaphus*) died with clinical symptoms suggestive of babesiosis. *Babesia* sp. organisms similar in morphology to *B. odocoilei* of white-tailed deer (*Odocoileus virginianus*) were observed in Giemsa-stained blood films from one of the elk. Continuous cultures of the parasite were established. Antiserum raised against the elk *Babesia* sp. isolate was compared to *B. odocoilei* specific antiserum in an immunofluorescent antibody assay; we found evidence of differences in reactivity to several *Babesia* spp. isolated from wildlife and domestic ruminants. Cultured parasites from the elk were not infective to either intact or splenectomized *Bos taurus* steers.

*Key words:* *Babesia*, in vitro culture, elk, *Cervus elaphus*.

*Babesia odocoilei* of white-tailed deer (*Odocoileus virginianus*) is the only *Babesia* of wildlife ruminants speciated in North America (Emerson and Wright, 1968, 1970), but recent isolates have been described from bighorn sheep (*Ovis canadensis nelsoni*), mule deer (*Odocoileus hemionus*), and a caribou (*Rangifer tarandus caribou*) (Goff et al., 1993; Thomford et al., 1993; Holman et al., 1994). Although babesiosis of white-tailed deer has been reported in free-ranging deer (Emerson and Wright, 1968), *Babesia* spp. infections appear to exist without causing serious disease problems (Perry et al., 1985). However, the increase in cervid ranching has resulted in the movement of many nondomestic hoofstock into areas where they are not normally found. This can be problematic to the new, susceptible animals as they enter an environment that may be endemically stable relative to disease agents in the native animals. Equally important is the possibility of introducing an exotic disease agent with the arrival of new animals. Stressful situations, such as when hoofstock are gathered and crowded together for management purposes, also may exacerbate carrier infections of transmissible disease agents. We report a *Babesia* sp. isolated from an elk (*Cervus elaphus*) raised under farming conditions with symptoms suggestive of babesiosis. This is the first reported description of a *Babesia* sp. from a North American elk.

Three 6 to 8-mo-old North American elk (*C. elaphus*) among approximately 110 adults and young enclosed on 101 ha near Del Rio, Texas (USA) (29°50′N, 102°10′W), died after a short illness. The first two deaths were of male animals and were notable because of extreme generalized icterus and the presence of numerous engorged ticks. The third elk, a female, died suddenly and on post mortem was found to have hydropericardium, hydroperitoneum, and edema of the mesentery with numerous engorged hemal nodes. The lungs were bright orange, but this elk was not noticeably icteric. Blood from this female drawn into ethylenediaminetetraacetic acid (EDTA) (Terumo Medical, Elkton, Maryland, USA) and several ticks removed from the elk and preserved in ethanol were submitted to Texas A&M University, College Station, Texas (TAMU) for examination. *Babesia* sp. was observed on the Giemsa-stained blood films. The ticks were identified as engorged adult female *Dermacentor albipictus* (Keirans and Litwak, 1989).

For parasite culture, the blood was prepared and cells dispensed into duplicate wells of a 24-well culture plate using the

methods of Holman et al. (1993b). Growth medium consisted of HL-1 medium (Hycor Biomedical Inc., Portland, Maine, USA) with 2 mM L-glutamine (Gibco, Grand Island, New York, USA), 20% normal adult bovine serum (from a donor Holstein steer housed at TAMU), and 500 U/ml penicillin, 500 μg/ml streptomycin, 125 μg/ml Fungizone (Gibco) and 100 μg/ml gentamicin (Schering Corporation, Kenilworth, New Jersey, USA) added. The culture plate was incubated at 37 C in a humidified atmosphere of 2% oxygen, 5% carbon dioxide and 93% nitrogen. The cultures were fed daily and subcultures done every 2 to 4 days dependent on growth as previously described (Holman et al., 1993b). Parasite growth was monitored by Giemsa-stained thin blood films.

Uninfected erythrocytes (RBC's) for culture were obtained from a zoo-housed (Minnesota Zoo accession 6557) adult North American elk (*C. elaphus*). The blood was collected by cephalic venipuncture into liquid EDTA and shipped overnight on ice to TAMU. Upon arrival, the blood was washed three times at 500 × *g* in 5 volumes phosphate buffered saline with 15 mM EDTA with complete removal of the buffy layer at each wash. The final pellet was resuspended in an equal volume of Puck's saline glucose (Gibco) with 20 g/l extra glucose (PSG + G) and stored at 4 C until use. Samples also were subjected to culture conditions as described previously to ensure they were *Babesia*-free.

Uninfected white-tailed deer (WTD) blood (kindly donated by Drs. Duane Kraemer and Mark Westhusin, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas) from a captive-reared adult female was collected by jugular venipuncture into EDTA. The erythrocytes (RBC's) were prepared for culture use as already described.

Cultured parasitized erythrocytes were cryopreserved in PSG + G containing 20% polyvinylpyrrolidone (PVP-40; Sigma, St. Louis, Missouri, USA) and subsequently were reestablished in vitro in WTD RBC's using the methods of Holman et al. (1988).

Two 2-yr-old *Bos taurus* cross-bred steers, one splenectomized and one spleen-intact, with no prior exposure to *Babesia* spp., were inoculated intramuscularly with the elk *Babesia* sp. Each animal received $10^8$ parasitized elk RBC's from a 48 hr culture (passage two). To calculate the inoculum, 1,000 total RBC's were counted on a Giemsa-stained slide and the percent parasitemia determined. Using a hemocytometer (Improved Neubauer counting chamber, Curtin Matheson Scientific, Inc., Houston, Texas), the RBC's/ml in the culture were counted. The culture volume equivalent to $10^8$ parasitized RBC's was calculated and then centrifuged at 500 × *g* for 15 min. The supernatant was removed and 0.5 ml PSG + G gently added over the cell pellet. The cells were kept on ice during transport to the animal holding area and were resuspended in the overlying PSG + G just prior to inoculation. Hematocrit samples and thin blood smears were prepared from each animal daily beginning on day 7 after inoculation and continuing through day 18. On day 18 the spleen-intact animal again was inoculated intramuscularly with $10^8$ parasitized RBC cryopreserved in 0.5 ml PSG + G containing 20% PVP. Six weeks later serum samples were obtained.

Antigen slides for the immunofluorescent antibody (IFA) test were prepared from cultured *B. odocoilei*, *B. bovis*, *B. divergens*, a *Babesia* sp. isolated from a bighorn sheep (Goff et al., 1993), a *Babesia* sp. isolated from a caribou (Holman et al., 1994), and the *Babesia* sp. isolated from the elk. *Babesia odocoilei* and the caribou, bighorn sheep and elk *Babesia* spp. were cultured in erythrocytes from the same white-tailed deer to minimize and equalize background fluorescence. Appropriate standard positive and negative control sera, preinoculation serum, and bovine anti-elk *Babesia* sp. serum collected 6 wk after the booster inoculation were tested at a dilution of 1:100 using the techniques of Goff

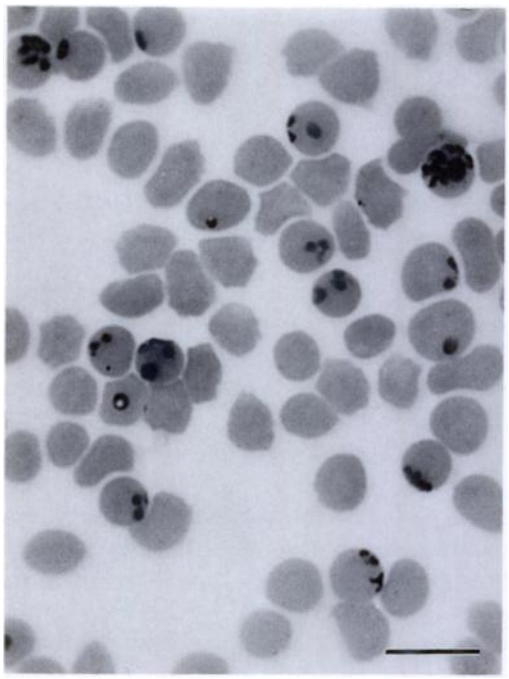

FIGURE 1. Giemsa-stained smear of the *Babesia* sp. from the elk cultured in elk erythrocytes. The parasite frequently was observed along the edge of the cell. Erythrocytes containing one, two, and multiple parasites are shown. Bar = 10 μm.

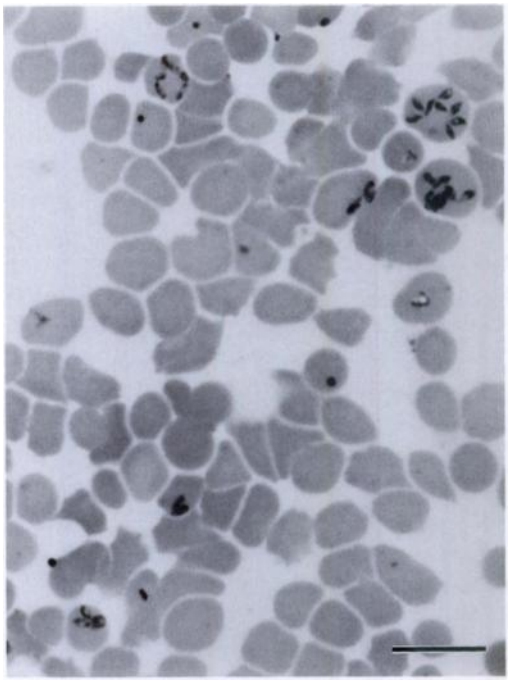

FIGURE 2. Giemsa-stained smear of the *Babesia* sp. from the elk cultured in white-tailed deer erythrocytes. The parasites often were located at the periphery of the erythrocyte, and single, paired, and multiply infected cells were observed. In white-tailed deer erythrocytes the parasites frequently appeared more pointed than those cultured in elk erythrocytes. Bar = 10 μm.

et al. (1982). Specific anti-*B. odocoilei* WTD serum and negative control WTD serum also were tested with all antigens at a 1:100 dilution.

A few *Babesia* sp. organisms were evident in the elk RBC cultures 24 hr after initiation. The percent parasitemia for 1,000 cells was 1.2 by 72 hr, and as many as eight parasites were observed within a single erythrocyte. On day 5, when the parasitemia was 4.4%, subcultures into RBC's from uninfected elk 6557 were made. The cultures were passed successively on days 7, 10, 12, 14 and 16. On days 13 and 14 (passage 6), lowered parasitemias and unhealthy appearing *Babesia* sp. organisms were observed; but following this brief crisis period, the parasites recovered and were subcultured every 48 hr at parasitemias ranging from 3 to 19%. Percent parasitemias >15% were frequent after the ninth passage and the parasites continued to thrive.

After establishment, the elk *Babesia* sp. was subcultured into WTD RBC's. The parasites grew in the WTD RBC's, and subcultures were made every 48 hr when the parasitemia was 7 to 13%. The *Babesia* sp. also was easily established in WTD RBC's after cryopreservation. Parasites first were evident 48 hr after resuscitation. By day 3 the parasitemia was 7.5% and the culture was successfully passed.

The *Babesia* sp. isolated from the elk was small and often located at the erythrocyte periphery in what is termed the accolé position (Fig. 1). Multiple parasites within a cell and dividing forms were frequently seen (Fig. 1). The stages observed were similar whether the parasite was cultured in elk or WTD RBC, but parasites in elk RBC appeared more rounded than those in WTD RBC (Figs. 1, 2). In general,

the parasites in elk RBC's appeared similar to *B. odocoilei* cultured in WTD RBC's (Holman et al., 1988).

Both steers inoculated with the elk *Babesia* sp. isolate exhibited a transient 4 to 5% drop in hematocrit values on day 11 after inoculation, but regained normal levels on day 12. No parasites were observed in stained blood smears from these animals at any time. The intact animal developed detectable antibodies by day 14 and serum collected 6 wk after the second exposure to parasites showed strong antibody activity to the elk *Babesia* sp. isolate. When tested by IFA for reactivity to other *Babesia* spp., this animal had no antibody activity to *B. bovis* or *Babesia* spp. from a caribou or a bighorn sheep (Table 1). A slight cross reaction was noted with *B. divergens*. Although the elk *Babesia* sp.-specific antiserum reacted with *B. odocoilei* antigen, the reaction was not as strong as that observed with the homologous antigen. In comparison, *B. odocoilei* specific antiserum reacted with the bighorn sheep *Babesia* sp. isolate, the elk isolate, and with *B. odocoilei*; slight cross reactions were observed with *B. bovis*, *B. divergens*, and the caribou *Babesia* sp. isolate.

Clark and Zetek (1925) observed a *Babesia* sp. in brain smears from a white-tailed deer in Panama. Subsequently, *Babesia capreoli* was reported in European roe deer (*Capreolus capreolus*), European red deer (*Cervus elaphus*), and sika deer (*Cervus nippon*) (Enigk and Friedhoff, 1962, 1963; Gray et al., 1991). *Babesia odocoilei* and a larger piroplasm have been described in white-tailed deer in the United States (Spindler et al., 1958; Emerson and Wright, 1968, 1970). Thus, recent descriptions of *Babesia* sp. isolates from other North American wildlife ruminants are not surprising (Goff et al., 1993; Thomford et al., 1993). However, since clinical babesiosis is not normally associated with wildlife, the isolation of a *Babesia* sp. from an elk exhibiting clinical signs suggestive of the disease is unexpected. The exact cause of death of the elk was not determined since the post mortem examination did not include histopathologal evaluations. Since the three elk that died were in a ranched herd, disconcerting questions are raised regarding the problems that infectious agents may cause, especially for native wildlife, when exotic hoofstock are introduced to new environments. Although some information on the epizootiology of tick-borne diseases in cervids in North America is available (Waldrup et al., 1992), more studies are needed for adequate risk assessment of these emerging diseases.

TABLE 1. Comparative reactivity of antiserum from an infected steer against the elk *Babesia* sp., and antiserum from an infected white-tailed deer against *Babasia odocoilei*, tested on ruminant *Babesia* spp. antigens in the immunofluorescent antibody test.

| Antigen | Antiserum | |
|---|---|---|
| *Babesia* sp./host | Anti-elk *Babesia* sp. | Anti-*B. odocoilei* |
| *Babesia* sp./elk | ++* | + |
| *B. odocoilei*/white-tailed deer | + | + |
| *Babesia* sp./bighorn sheep | − | + |
| *Babesia* sp./caribou | − | ± |
| *B. bovis*/cattle | − | ± |
| *B. divergens*/cattle | ± | ± |

* ++, very strong reaction (titer > 1:100); +, strong reaction (titer = 1:100); ±, cross reaction (titer < 1:80); and −, no reaction at 1:80 dilution.

The advantages of culture techniques in the study of *Babesia* spp. are apparent. Quantities of parasites can be raised without necessitating splenectomizing and infecting hosts; in the scenario of a *Babesia* sp. isolated from an exotic ruminant, a more common source of erythrocytes may well serve in vitro. The difficulty in obtaining elk blood to continually culture this isolate, for example, led to the successful use of white-tailed deer erythrocytes. In addition, a number of morphologically similar wildlife *Babesia* spp. isolates cultured in erythrocytes from the same donor white-tailed deer provided standardized IFA antigens for use in this study and cultured parasites were used to raise specific bovine antiserum.

The successful propagation of the elk

*Babesia* sp. isolate in white-tailed deer erythrocytes would seem to support the possibility that this isolate is *B. odocoilei*. However, other *Babesia* spp. can use white-tailed deer erythrocytes in vitro as well. It has been shown that *B. bovis* can be cultivated in white-tailed deer erythrocytes (Holman et al., 1993a), although white-tailed deer are refractory to infection by *B. bovis* (Kuttler et al., 1972).

Based on the IFA assay, this isolate is distinct from the *Babesia* spp. isolated from a caribou and a bighorn sheep, but appears to share antigens with *B. odocoilei*. However, while the *B. odocoilei* antiserum cross reacted strongly with the bighorn sheep *Babesia* sp. antigen, the elk *Babesia* sp. antiserum showed no reactivity to this antigen. We conclude that the elk was not infected with native *B. bovis* since the IFA assay for antibody activity to *B. bovis* was negative, the elk isolate was morphologically distinct from *B. bovis*, and since both spleen-intact and splenectomized *Bos taurus* steers were not susceptible to infection with the elk *Babesia* sp. isolate. Further studies are underway to clarify the relationship of this isolate with other *Babesia* sp. isolates of wild and domestic ruminants.

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