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# EXPERIMENTAL INFECTION OF CAPTIVE AXIS DEER WITH BRUCELLA ABORTUS

## D. S. Davis,<sup>1</sup> F. C. Heck,<sup>2</sup> and L. G. Adams<sup>3</sup>

ABSTRACT: Four captive-raised axis deer, Axis axis (Erxleben), which were negative serologically to Brucella were inoculated with  $1 \times 10^{\circ}$  virulent Brucella abortus biotype 1 organisms (Texas #221 isolate) administered bilaterally into the conjunctival sac. Sera collected from each deer prior to inoculation and 30 days post-inoculation (PI) were examined for Brucella antibodies by the buffered Brucella antigen (card), the rivanol precipitation, the standard tube agglutination, and the cold complement fixation tube serologic tests. All four axis deer converted serologically as determined by all tests at 30 days PI. Brucella abortus biotype 1 was isolated from 26 of 32 tissue samples collected at necropsy and also from milk from the lactating female.

#### INTRODUCTION

The southwestern region of the United States and Texas in particular is occupied by an increasing number of exotic or nonnative game species. These exotic species are generally of African or Asian origin but game animals from Europe are also present. As determined by the Texas Parks and Wildlife Department (Harmel, 1980), the exotic game population in Texas has increased from an estimated 13,000 in 1963 to more than 72,000 animals in 1979 and the number of exotic species from 13 to 51. The exotic game species are, in some cases, confined or enclosed in areas surrounded by "game-proof" fences. About one-third of the total number of the major exotic species, however, are free-ranging and occur in over 64 counties in the state (Harmel, 1980). These free-ranging exotics feed on and occupy areas with native game and domestic livestock. The health status and disease reservoir potential of these free-ranging and confined exotic species are not well known.

Axis deer are the most abundant and

widespread non-native deer in Texas with population estimates in 1979 exceeding 22,000. Free-ranging axis deer occur in 20 counties, and in 59 counties axis deer are confined within "game-proof" fenced pastures (Harmel, 1980). In most cases the axis deer are held with other exotics, native game, and domestic livestock under crowded conditions conducive to intraspecific and interspecific transmission of diseases.

Brucellosis is a disease of economic, political, and public health importance in Texas. Several species of deer occurring in Texas have been shown to be either naturally or experimentally susceptible to Brucella infection. White-tailed deer (Odocoileus virginianus Zimmermann) were shown to be susceptible experimentally to B. abortus (Youatt and Fay, 1959; Baker et al., 1962), and serologic evidence indicates that on rare occasions they may be naturally exposed (Steen et al., 1955; Shotts et al., 1958; Youatt and Fay, 1959; Hayes et al., 1960; Trainer and Hanson, 1960). Only once has B. abortus been isolated from a wild white-tailed deer (Corey et al., 1964). Mule deer (Odocoileus hemionus (Rafinesque)) have also been shown to be experimentally susceptible to infection with B. abortus (Thorpe et al., 1967), and serologic evidence of natural exposure has been documented (Thorpe et al., 1965). McDiarmid (1951) found agglutinins to B. abortus in the sera of sika deer (Cervus nippon (Temminck)) and

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fallow deer (*Dama dama* (L.)). Rocky Mountain elk (*Cervus elaphus nelsoni* Bailey) in North America have been shown by both serologic and bacteriologic examinations to be infected naturally with *B. abortus* (Tunnicliff and Marsh, 1935; Corner and Connell, 1953; Adrian and Keiss, 1977; Thorne et al., 1978).

The effects of exposure of axis deer to *Brucella* have not been established. The purpose of the present investigation is to determine the susceptibility of axis deer to *B. abortus* infection, their serologic response, and their reservoir potential as non-bovine hosts for *B. abortus*.

#### MATERIALS AND METHODS

Four captive axis deer (three adult does, one yearling male) that had been raised in isolation on the Research Park of the College of Veterinary Medicine, Texas A&M University were used in this study. The deer were shown to be serologically negative for *Brucella* antibodies as determined by the buffered *Brucella* antigen (card), the rivanol precipitation (Riv), the standard tube agglutination (STA) (as described in the National Animal Disease Laboratory Diagnostic Reagents Manual 650 E and F), and the cold complement fixation tube (CCFT) (Jones et al., 1963) tests.

The axis deer were immobilized with xylazine hydrochloride, (2-3 mg/kg Rompun-xylazine HCl, Bayvet Division, Cutter Laboratories, Inc., Shawnee, Kansas 66231, USA) and inoculated individually with 100  $\mu$ l of a PBS solution containing  $1 \times 10^{\circ}$  virulent B. abortus biotype 1 organisms of bovine origin (Texas #221 isolate) administered bilaterally into the conjunctival sacs (50  $\mu$ l each). Blood samples were collected from each deer via jugular venipuncture immediately prior to Brucella exposure and 30 days post-inoculation (PI). The deer were killed at 30 days PI and tissue samples were collected at necropsy and held at -20 C until inoculated on Farrell's medium (Farrell, 1974). Comparable tissue samples were fixed in 10% buffered formalin, paraffin embedded, sectioned at  $4 \,\mu m$ and stained with hematoxylin and eosin.

#### RESULTS

All four axis deer were negative serologically for *Brucella* antibodies on the day of inoculation as indicated by all methods utilized. At 30 days PI, sera from all the

TABLE 1. Serologic reactions of four axis deer 30 days post-inoculation with  $1 \times 10^8$  Brucella abortus organisms.<sup>4</sup>

Deer no.		Sex	Card⁵	Serum titer			
	Age			STA	RIV <sup>d</sup>	CCFT.	
1	6 yr	F	Pos.	200	200	1 + 80'	
2	4 yr	F	Pos.	200	200	1 + 80	
3	3 yr	F	Pos.	200	200	3 + 40	
4	l yr	Μ	Pos.	100	200	4 + 80	

• All deer were seronegative for *B. abortus* antibodies by all tests prior to inoculation.

<sup>b</sup> Card-buffered Brucella antigen test.

' STA = standard tube agglutination test

<sup>d</sup> RIV-rivanol precipitation test.

CCFT = cold complement fixation tube test.

'1 + 80 = a 1 + reaction observed at a serum dilution of 1:80.

deer were *Brucella* reactive at levels considered positive by criteria for bovine brucellosis (card positive,  $Riv \ge 1:100$ ,  $STA \ge 1:100$ ,  $CCFT \ge 1:40$ ) (Table 1).

Brucella abortus biotype 1 was isolated from 26 of 32 tissue samples collected at necropsy and also from milk collected from a lactating doe (Table 2).

Morphologic evaluation of the reproductive systems failed to disclose significant lesions. The suprapharyngeal, mandibular, internal iliac, and inguinal or supramammary lymph nodes were moderately hypertrophic and hyperplastic, with large numbers of hypercellular mature secondary germinal centers and cellular expansion of the paracortex.

## DISCUSSION

The data indicated that axis deer were readily infected when exposed to *B. abortus.* Serologic reactions observed in the deer at 30 days PI were similar to those seen in susceptible bovine hosts. If the current bovine criteria for classification were applied, all of the axis deer would be declared as *Brucella* reactors by any of the serologic tests.

The frequency of *B. abortus* isolations from the tissues equals or surpasses what one would expect in infected cattle. *Bru*-

	Deer number					
Tissue/sample	1	2	3	4		
Liver	+	+	+	+		
Spleen	+	+	+	+		
Suprapharyngeal L.N.*	+	+	+	+		
Prescapular L.N.	+	+	+	+		
Parotid L.N.	+	+	+	+		
Supramammary L.N.	-	+	+	+ Þ		
Internal iliac L.N.	+	+	+	-		
Uterus	-	-	-	NA		
Testes	NA <sup>c</sup>	NA	NA	-		
Urine	NA	-	-	-		
Feces	-	-	-	-		
Milk	NA	+	_	NA		

 TABLE 2.
 Isolations of *B. abortus* biotype 1 from axis deer samples collected at necropsy 30 days post-inoculation.

• L.N. = lymph node

<sup>b</sup> Inguinal lymph node.

° Not available.

cella abortus was isolated from 81% (26 of 32) of the deer samples collected and cultured. Isolations of *B. abortus* from milk and uterus from deer #2 indicated that vertical transmission was possible in axis deer. The histologic lesions observed in axis deer tissues were compatible with broad spectrum polyclonal local and systemic antigenic stimulation by a bacterium such as *B. abortus*.

The prevalence of *Brucella* infections in free-ranging populations of axis deer is unknown; however, the species may represent a suitable non-bovine, exotic wildlife reservoir for *B. abortus* if this organism is introduced into populations of this widespread, non-native game animal.

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