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Source: Journal of Wildlife Diseases, 21(2) : 132-136

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-21.2.132

Journal of Wildlife Diseases, 21(2), 1985, pp. 132–136

BRUCELLA ABORTUS IN WILDLIFE ON SELECTED CATTLE FARMS IN ALABAMA

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ABSTRACT: Two studies of brucellosis in wildlife on farms where the brucellosis infection prevalence in cattle was known are reported. On a research farm, 233 feral animals of 22 mammalian species and 12 of seven avian species were trapped during three time periods. Sixty were studied before cattle were introduced, 128 were studied while 501 cattle infected with *Brucella abortus* were calving and aborting, and 60 specimens were collected 20 mo after the last infected cow calved. Selected tissues from 229 wild animals were cultured and sera from 138 were examined using the brucellosis card, standard tube agglutination (STA), 2-mercaptoethanol (2-ME) and rivanol (RIV) tests. *Brucella abortus* was not recovered from any animals sampled prior to cattle being introduced and all sera collected were negative. *Brucella abortus* was isolated from four opossums (*Didelphis virginiana*) and one raccoon (*Procyon lotor*) in the group of animals trapped during the calving period. Three serums were tested and had STA titers ranging from 1:100 to 1:200. Of 68 sera only one had antibodies. *Brucella* were not isolated from 59 animals trapped after the calving period and only one of 42 serums had antibodies. On regional cattle farms, 243 wild animals were trapped. Brucellae were not isolated from 223 animals which were cultured. No serums had significant titers. The data from this study suggest opossums and raccoons can be infected from cattle but are unlikely to maintain the infection.

INTRODUCTION

Numerous serologic surveys and bacteriologic studies have demonstrated that *Brucella abortus* can infect various species of wildlife. Relatively few studies have attempted to determine the prevalence of wildlife brucellosis in areas where the brucellosis infection prevalence in cattle is known (Moore and Schnurrenberger, 1981a). Two prevalence studies of brucellosis in wild animals were conducted: (1) on a research farm before, during, and after possible natural exposure to *B. abortus*-infected cattle, and (2) on cattle farms in an area where the herd infection prevalences were between 0.25 and 1.5%.

MATERIALS AND METHODS

Wild animals were trapped on a research farm in east central Alabama during three time periods: before cattle were introduced, while *B. abortus*-infected cattle were aborting and calving, and 20 mo after the last infected cow calved. A herd of 648 infected cows that included 501 pregnant animals was assembled on a 160 ha farm. Herd assembly began in July 1977 and the last pregnancy terminated in May 1981 (Fig. 1). A total of 290 cows gave birth to live calves, and an additional 209 cows aborted or had dead calves. Cervical swabs and milk samples were obtained at the termination of each pregnancy and cultured. *Brucella abortus* biotype 1 was isolated from 309 cows, biotype 2 from 16, and biotype 4 from 24. Isolates from 55 other cows were not typed. The cows were retained for as long as 8 mo after termination of pregnancy.

Specimens from 233 feral animals, representing 22 mammalian species and 12 from seven avian species, were collected on the research farm during three time periods (Table 1). Sixty

Received for publication 29 September 1983.

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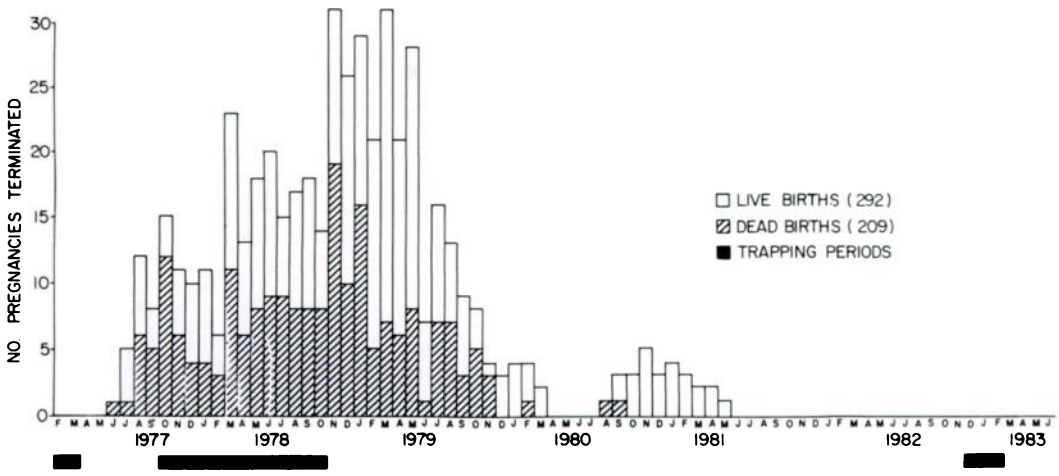

FIGURE 1. Temporal relationship between wildlife trapping periods and pregnancy termination of cattle infected with *Brucella abortus* in Alabama.

animals were trapped during the preexposure period of October 1977 through October 1978 and 60 postexposure during December 1982 through February 1983 (Fig. 1). The retropharyngeal lymph nodes, lungs, kidneys, gonads, spleen and liver were collected from each animal plus mammary tissue and fetuses from females, if available, and frozen (−70 C) until cultured. Each tissue was dipped briefly in 95% ethanol, flamed, and incised with a sterile surgical blade. The open surface was macerated with a scalpel and spread on two plates of tryptose agar containing 5% bovine serum and antibiotics (Alton et al., 1975). The plates were incubated in a humidified atmosphere of air of 8% CO_2 at 37 C and examined for colonies after 3 and 7 days. Isolates of *Brucella abortus* were identified and biotyped using standard serologic and biochemical criteria (Alton et al., 1975). Blood samples were obtained from 23 animals preexposure, 73 during the exposure period, and 42 postexposure (Table 1). Sera were examined using the brucellosis card (Brucellosis Card Test, Hynson, Westcott and Dunning, Baltimore, Maryland 21202), standard tube agglutination (STA), 2-mercaptoethanol (2-ME) and rivanol (RIV) tests (Alton et al., 1975; USDA-ARS).

Additional studies were conducted on 243 wild animals of nine species which were trapped on cattle farms 16 to 40 km from the brucellosis research farm (Table 2). The brucellosis infection prevalences in the cattle herds were between 0.25 and 1.5%. The retropharyngeal lymph nodes, gonads and spleen from 223 animals were cultured and serums from 100 animals were tested using card, STA, 2-ME and RIV methods.

RESULTS

On the research farm, 60 animals were trapped during the preexposure period (Table 1). The 54 animals which were cultured and the 23 whose serums were tested were negative. In those tested during the calving period, *B. abortus* biotype 1 was isolated from four of 14 opossums and from one of six raccoons (Table 3). Serums of two of the four culture positive opossums and the culture positive raccoon were tested. One opossum and the raccoon were card test positive. The STA and 2-ME test titers ranged from 1:100 to 1:200. The RIV titers ranged from 1:50 to 1:100. The other 105 mammals and birds cultured during the calving period of the cattle were negative. Only one of the remaining 70 serums had a low titer. One culture negative coyote had a negative STA test. A culture negative gray fox had an incomplete reaction in the 1:50 STA dilution. Of the 60 animals trapped during the postexposure period, 59 were cultured and 42 serums were tested. All were culture negative. Serum from a raccoon had an incomplete reaction in the 1:50 dilution on STA and 2-ME tests.

Of the 243 feral animals from regional cattle farms, 223 were cultured and 100 serums were tested. All were negative.

TABLE 1. Wild and feral animals collected from the research farm in Alabama from which tissues were cultured and/or sera tested.

Species	No. collected			No. cultured			No. serotested		
	Pre[a]	Exp[b]	Post[c]	Pre	Exp	Post	Pre	Exp	Post
Opossum (*Didelphis virginiana*)	13	16	17	13	14 (4)[d]	16	10	12 (2)[e]	17
Short-tailed shrew (*Blarina brevicauda*)	1	1	1	1	1	1	0	0	0
Cottontail rabbit (*Sylvilagus floridanus*)	0	3	0	0	3	0	0	1	0
Chipmunk (*Tamias striatus*)	0	1	0	0	1	0	0	0	0
Gray squirrel (*Sciurus carolinensis*)	0	2	0	0	2	0	0	0	0
Beaver (*Castor canadensis*)	0	1	0	0	0	0	0	0	0
Rice rat (*Oryzomys palustris*)	3	9	2	3	9	2	0	5	1
White-footed mouse (*Peromyscus leucopus*)	5	1	0	5	1	0	1	0	0
Cotton rat (*Sigmodon hispidus*)	8	42	10	6	41	10	0	29	2
Muskrat (*Ondatra zibethicus*)	2	0	0	2	0	0	0	0	0
House mouse (*Mus musculus*)	5	20	5	5	19	5	0	8	0
Norway rat (*Rattus norvegicus*)	0	1	0	0	1	0	0	1	0
Roof rat (*Rattus rattus*)	6	0	0	6	0	0	0	0	0
Feral dog (*Canis familiaris*)	1	0	1	0	0	1	1	0	1
Coyote (*Canis latrans*)	0	2	0	0	2	0	0	2	0
Gray fox (*Urocyon cinereoargenteus*)	6	9	5	4	7	5	6	7 (1)[e]	5
Raccoon (*Procyon lotor*)	5	7	13	5	6 (1)[d]	13	5	4 (1)[e]	12 (1)[e]
Mink (*Mustela vison*)	0	0	1	0	0	1	0	0	0
Spotted skunk (*Spilogale putorius*)	1	0	1	1	0	1	0	0	0
Feral cat (*Felis domestica*)	0	1	3	0	1	3	0	0	3
Bobcat (*Felis rufus*)	0	0	1	0	0	1	0	0	1
White-tailed deer (*Odocoileus virginianus*)	0	1	0	0	0	0	0	1	0
Turkey vulture (*Cathartes aura*)	0	1	0	0	1	0	0	1	0
Bobwhite (*Colinus virginianus*)	1	2	0	1	2	0	0	0	0
American crow (*Corvus brachyrynchus*)	1	2	0	1	2	0	0	2	0
Blue jay (*Cyanocitta cristata*)	1	1	0	0	1	0	0	0	0
Brown thrasher (*Toxostoma rufum*)	0	1	0	0	1	0	0	0	0
Robin (*Turdus migratorius*)	1	0	0	1	0	0	0	0	0
European starling (*Sturnus vulgaris*)	0	1	0	0	1	0	0	0	0
Total no.	60	125	60	54	116 (5)	59	23	73 (4)	42 (1)

TABLE 2. Wild animals collected from cattle farms at least 16 km from the research farm in Alabama from which tissues were cultured and serums tested.

Species	No. collected	No. cultured	No. tested*
Opossum (*Didelphis virginiana*)	57	57	36
Beaver (*Castor canadensis*)	24	24	1
Muskrat (*Ondatra zibethicus*)	78	78	13
Red fox (*Vulpes fulva*)	1	0	1
Gray fox (*Urocyon cinereoargenteus*)	27	9	21
Raccoon (*Procyon lotor*)	45	44	25
Mink (*Mustela vison*)	8	8	0
River otter (*Lutra canadensis*)	1	1	1
Bobcat (*Felis rufus*)	2	2	2
Total no.	243	223	100

* All serums were negative.

DISCUSSION

The isolation of *B. abortus* from four opossums and a raccoon (Swann et al., 1980) which were trapped on the research farm during the exposure period demonstrated that transmission of infection from cattle to wildlife is possible. The infections probably resulted from scavenging infected fetuses and placentae. The inability to isolate *B. abortus* from these species after the period when cattle were shedding and the fact that only one serum was positive in raccoons over 1 yr of age suggest that they are unlikely to maintain the infection within a population through intraspecies transmission. The inability to isolate *B. abortus* from wildlife which were trapped on area cattle farms suggests that infection is rare if exposure is relatively low.

In an earlier study, *B. abortus* was isolated from three of 23 opossums trapped from east central Alabama (Moore and Schnurrenberger, 1981b). Of three culture positive opossums, two were negative on serologic tests. Experimental exposure of opossums confirmed the susceptibility of this species and demonstrated that they

TABLE 3. Bacteriologic and serologic test results from culture positive and sero-reactive wild animals in Alabama. Titers of STA, 2-ME and RIV tests are expressed as the reciprocal of the final dilution showing agglutination.

Species	Sex	Culture	Serologic tests			
			CARD[a]	STA[b]	2-ME[c]	RIV[d]
Opossum[e]	Female	+	ND	ND	ND	ND
Opossum[e]	Male	+	ND	ND	ND	ND
Opossum[e]	Male	+	+	100	100	50
Opossum[e]	Male	+	−	200	200	100
Gray fox[e]	Male	−	−	150	—	—
Raccoon[e]	Male	+	+	200	100	100
Raccoon[f]	Male	−	−	150	150	—

[a] Brucellosis card test.
[b] Standard tube agglutination test.
[c] 2-Mercaptoethanol test.
[d] Rivanol test.
[e] Trapped on research farm during the exposure period, October 1977 through October 1978.
[f] Trapped on research farm during the postexposure period, December 1982 through February 1983.

←

[a] Preexposure period, February and March 1977.
[b] Exposure period, October 1977 through October 1978.
[c] Postexposure period, December 1982 through February 1983.
[d] No. of culture positive animals in parentheses.
[e] No. of sera reactive to one or more tests in parentheses.

could remain infected for at least 19 wk. The studies suggested opossums were unlikely to shed organisms except through short-term mechanical carriage in the intestinal tract. Since only three raccoons have been cultured (Boerr et al., 1980) and 30 serums tested with only one positive on the card test and another with a 1:25 STA titer (Randhawa et al., 1977; Hoq, 1978; Boerr et al., 1980), it is difficult to evaluate their potential as reservoirs. No experimental infections of raccoons have been reported. Experimental infections of opossums (Moore and Schnurrenberger, 1981b) and gray foxes (Scanlan et al., 1984) plus field studies of elk (*Cervus elaphus nelsoni*) (Lee and Turner, 1937; Morton et al., 1981) and coyotes (Davis et al., 1979) raise serious doubts on the accuracy of standard serologic tests for brucellosis when applied to wild animal species. Although many species of wildlife can become infected with *Brucella abortus*, there is little evidence that wildlife transmit the disease to cattle.

ACKNOWLEDGMENTS

This study was supported as Agricultural Experiment Station Project ALA-11-0052 and Cooperative Agreement 12-16-4-341 between USDA/APHIS and Auburn University. Publication No. 1630 School of Veterinary Medicine and No. 5-83455 Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama 36849, USA.

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