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Paratuberculosis in Free-Ranging Fallow Deer in Spain

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ABSTRACT: Paratuberculosis was diagnosed in a population of approximately 1,000 free-ranging fallow deer (*Dama dama*) sampled from 1997–98 in the Regional Hunting Reserve of El Sueve (Asturias, Spain). Five of eight animals observed with diarrhea were diagnosed as having paratuberculosis on the basis of gross lesions at postmortem examination and histopathology. In two deer, *Mycobacterium avium* subsp. *paratuberculosis* was cultured and identified by polymerase chain reaction. Indirect enzyme-linked immunosorbent assay and immunodiffusion tests were used to evaluate sera from 33 adult deer from this population. All fallow deer tested were seronegative.

Key words: Dama dama, fallow deer, *Mycobacterium avium* subsp. *paratuberculosis,* paratuberculosis, Spain.

Paratuberculosis (Johne's disease) is a chronic disease of domestic and wild ruminants with a widespread distribution. It is caused by *Mycobacterium avium* subsp. *paratuberculosis* which proliferates in the mucosa of the terminal ileum, cecum, colon, and adjacent lymph nodes. It is characterized by loss of weight and body condition. Paratuberculosis is well documented in cattle, sheep, and goats but there are relatively few reports in free-ranging deer (Jessup and Williams, 1999). Most cases in non-domestic ruminants occur in captive animals and a wide variety of species have been involved (Weber and Gürke, 1992; Fawcett et al., 1995; Manning and Collins, 1999). *Mycobacterium avium* subsp. *paratuberculosis* has been found in non-ruminant species suggesting that the epidemiology of this disease is more complex than previously realized (Beard et al., 2001). This paper describes findings of a 2 yr study of paratuberculosis in fallow deer (*Dama dama*). We evaluated health status and established a tracking program for diseases of the fallow deer population in the National Hunting Reserve of El Sueve in 1997–98 at the request of the Principado de Asturias Government.

The Regional Hunting Reserve of El Sueve (Principado de Asturias, in northern Spain; 43°15′N, 5°15′E) is a mountainous area of 8,300 ha, 5 km from the seashore. Fallow deer were introduced in 1960. Since then the population has increased so that population reduction was necessary for Reserve management. The population of fallow deer was estimated to be approximately 1,000 individuals in 1999. During summer there were about 1,200 cattle, 550 horses, and 850 sheep and goats that shared pastures and water holes with the deer.

Four adult males and four yearlings (three males and one female) observed with diarrhea were culled and subjected to a full postmortem examination and routine histologic study. Samples of the terminal ileum and associated mesenteric lymph nodes were fixed in neutral buffered 10% formalin, processed routinely, and stained with hematoxylin and eosin. Tissues also were stained with Ziehl-Nielsen acid-fast stain. Intestinal and lymph node samples from four animals were homogenized, decontaminated, and inoculated on Lowestein-Jensen medium, with and without mycobactin. Colonies obtained from culture with mycobactin were subjected to extraction, purification, and polymerase chain reaction (PCR) as described by Garrido et al. (2000) for *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum.*

Serologic screening was performed on 33 blood samples collected from hunted animals in the Reserve, including the eight fallow deer with diarrhea. Blood samples were taken by cardiac venipuncture immediately after death and sera were frozen

until processed. Two serologic techniques with PPA-3 antigen (Allied Monitor, Inc., Fayette, Missouri, USA) were used: an agar gel immunodiffusion test (AGID) (Allied Monitor, Inc.) according to directions of the manufacturer, and an indirect enzyme-linked immunosorbent assay (ELISA), with a specific protein G for ruminant IgG at a concentration of 0.05 ug/ml (Sigma, Alcobendas, Madrid, Spain), as described for sheep by Garrido et al. (1999). Paratuberculosis was suspected in five (one yearling and four adult males) of the eight fallow deer studied on the basis of gross lesions and histopathology. They were alert prior to being culled. These deer had diarrhea, and perineums were stained with liquid green feces. On necropsy, two were emaciated. Gross findings in all five animals were similar: enlargement of the ileocecal and mesenteric lymph nodes and different degrees of thickening and roughening of the mucosa of the distal small intestine and ileocecal valve.

On histologic examination, there was moderate to severe granulomatous enteritis with large numbers of small acid-fast organisms primarily in the cytoplasm of macrophages. The lamina propria of the distal small intestine was moderately to massively infiltrated with mononuclear cells. However, giant cell formation was uncommon. The mesenteric and ileocecal lymph nodes contained activated lymphoid follicles and granulomatous infiltrates. Acid-fast bacteria were observed in intestinal and lymph node samples from all five fallow deer.

Tissue samples from two fallow deer suspected to have paratuberculosis were available for culture and identification by PCR. Mycobacteria were isolated from ileocecal valve and associated lymph node of an adult male fallow deer and from terminal ileum of another adult male. Isolates were identified as *M. avium* subsp. *paratuberculosis* on the basis of mycobactin dependence and by PCR which was positive for *M. avium* subsp. *paratuberculosis* and negative for *M. avium silvaticum.* Tissue samples from two of the three fallow deer without macroscopic and microscopic lesions also were obtained for culture but results were negative. Sera from all animals, including the two infected, were negative for antibodies.

Because microscopic examination and bacterial culture detected the organism in two cases tested, we believe that the acid-fast rods observed histologically in the other three fallow deer also were also *M. avium* subsp. *paratuberculosis.*

Paratuberculosis has been described as one of the most prevalent and costly diseases affecting dairy cattle today and is a common disease in captive wild ruminants (Manning and Collins, 1999). However, it is rare in free-ranging wildlife (Jessup and Williams, 1999). In Europe, paratuberculosis has been diagnosed in captive fallow deer (Commichau, 1982), but to our knowledge this is the first report of the disease in a free-ranging population of fallow deer in Europe.

Clinical and pathologic manifestations of paratuberculosis in the fallow deer of our study were consistent with those in other deer species, although some differences were apparent. Severe progressive cases of paratuberculosis have been described in young (1–2 yr old) captive fallow deer, so that it appears that clinical disease is more common in young wild ruminants than in young cattle (Jessup and Williams, 1999). However, in our study, only one fallow deer was a yearling. Necrosis and mineralization of lesions described in some red deer (*Cervus elaphus*) and other wild ruminants (Williams et al., 1983; Jessup and Williams, 1999) were not observed in these cases.

Serologic tests were poor indicators of infection. Serologic tests used for domestic ruminants have not been extensively tested in wild ruminants, so there is no information on sensitivity and specificity in these species (Jessup and Williams, 1999). In our fallow deer, failure of the ELISA could be attributed to inappropriate con-

jugates for fallow deer. Results of the AGID test are more difficult to explain because it does not rely on species-specific immunoglobulins and usually show good sensitivity in clinical cases of paratuberculosis in cattle and, possibly, sheep. One explanation is that the immune response of fallow deer to infection falls in a different point of the mycobacterial immunopathologic spectrum.

Our results indicate that paratuberculosis is present in this geographic region. Cases of paratuberculosis have been recorded in domestic ruminants in this area, although there are no studies on the prevalence of the disease. Wild ruminants may become infected by contact with diseased domestic stock (Riemann et al., 1979), and transmission from deer to cattle has been suspected (Chiodini and Van Kruiningen, 1983). Therefore, it is possible that domestic ruminants were the source of infection for our fallow deer.

It is difficult to ascertain the effect of paratuberculosis on the fallow deer population in the Regional Hunting Reserve of El Sueve. In some wild ruminant populations, there is a high rate of subclinical infection and the effect of chronic infection can be determined only through long-term research (Jessup and Williams, 1999). However, it is unlikely that a wild animal population is severely affected because of relatively low virulence of the organism and low mortality. Attempts to eradicate the disease in free-ranging populations have never been successful because of its chronicity, environmental resistance of the causative agent, and lack of efficacious diagnostic tests, so prevention of infection is important (Jessup and Williams, 1999). The only options for management of infected herds are to decrease their density and not to use animals for translocation. Both measures have been implemented in this area. Vaccination, widely used in sheep in Spain, could also be considered if paratuberculosis seriously threatened wildlife populations, which is not the case for the fallow deer in Spain. Due to the complex nature of the disease and problems associated with infection of free-ranging deer eradication is unlikely in the near future.

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