SEROLOGICAL INVESTIGATIONS FOR SOME BACTERIAL AND VIRAL PATHOGENS IN FALLOW DEER (CERVUS DAMA) AND WILD BOAR (SUS SCROFA) OF THE SAN ROSSORE PRESERVE, TUSCANY, ITALY

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Source: Journal of Wildlife Diseases, 24(1) : 127-132

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.1.127

Journal of Wildlife Diseases, 24(1), 1988, pp. 127–132

SEROLOGICAL INVESTIGATIONS FOR SOME BACTERIAL AND VIRAL PATHOGENS IN FALLOW DEER (*CERVUS DAMA*) AND WILD BOAR (*SUS SCROFA*) OF THE SAN ROSSORE PRESERVE, TUSCANY, ITALY

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ABSTRACT: Sera of 43 fallow deer (*Cervus dama*) of the San Rossore Preserve (Tuscany, Italy) were examined for antibodies against eight pathogens; one proved positive for *Brucella* sp., 21 for *Listeria monocytogenes*, 34 for *Chlamydia psittaci*, three for *Coxiella burnetii*, one for infectious bovine rhinotracheitis virus, 11 for parainfluenza-3 virus, 25 for bovine viral diarrhea virus and six for bovine respiratory syncytial virus. No age and sex difference in the positivity rates and titers was evidenced, while a sex difference was found both in rates of infection and in titers against parainfluenza-3 virus. Parainfluenza-3 infection was more prevalent in 1984 than in 1983 sampling. Sera of 20 wild boars (*Sus scrofa*) of the same preserve were examined for antibodies against five pathogens: four sera were positive for *Brucella* sp., while all were negative for *Listeria monocytogenes*, *Chlamydia psittaci*, *Coxiella burnetii* and Aujeszky's disease virus. Public and animal health involvement with these diseases are discussed for these respective host species.

Key words: Serology, fallow deer, *Cervus dama*, wild boars, *Sus scrofa*, bacterial diseases, viral diseases, survey.

INTRODUCTION

Wildlife in Italy frequently poses problems related to the coexistence of wild animals with farming and human settlements in restricted areas. Therefore, management should consider also public and animal health problems (Mantovani and Leporati, 1976). In spite of a need for information on diseases, only one survey to determine the occurrence of bacterial and viral pathogens common to either domestic animals or humans has been performed in wild ungulates (Corradini and Pecorari, 1981). These authors tested 14 fallow deer (*Cervus dama*) and 23 red deer (*Cervus elaphus*) sera from Mesola Preserve (Po Valley, Italy), all of which were negative for antibodies to *Brucella abortus*, *Francisella tularensis*, and 11 serotypes of *Leptospira interrogans*; while almost all were positive for antibodies to *Listeria monocytogenes*, *Coxiella burnetii* and *Chlamydia psittaci*. An investigation to determine the prevalence of some selected pathogens of domestic animals in populations of fallow deer and wild boar (*Sus scrofa*) at San Rossore Preserve (Tuscany, Italy) was initiated.

Fallow deer sera have been tested for evidence of antibodies to eight pathogens found in cattle and/or domestic sheep in Italy. These included *B. abortus* (Caporale et al., 1980; Ghilardi et al., 1981), *L. monocytogenes* (Turilli et al., 1982; Sacco, 1985), *C. psittaci* (Andreani et al., 1983; Turilli et al., 1984), *C. burnetii* (Moretti, 1984), infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI-3), bovine viral diarrhea (BVD) and bovine respiratory syncytial (BRS) viruses (Cancellotti et al., 1980, 1984; Cancellotti and Carlotto, 1987).

Wild boar sera have been tested for antibodies against two pathogens commonly found in domestic swine in Italy, *L. monocytogenes* (Sacco, 1985) and Aujeszky's disease (Frescura et al., 1982). *Brucella suis* was never isolated in Italy. However, antibodies against *Brucella* sp., *C. psittaci* and *C. burnetii* were determined also to compare the prevalences of infection in the two wild species, although these three pathogens seem to be of no importance in domestic pig populations in Italy.

MATERIALS AND METHODS

Study area

In the last 3 yr the game management in San Rossore has been under the supervision of the Istituto Nazionale di Biologia della Selvaggina

TABLE 1. Antigen strains, tests employed and threshold titers for serological studies in fallow deer and wild boars in Italy.

Agent	Test	Positive threshold
Brucella abortus (S 19)	plate agglutination test[a]	30 IU
Brucella abortus (S 19)	complement fixation test	20 CFU
Chlamydia psittaci (heat inactivated antigen)	complement fixation test	1:16
Coxiella burnetii (Behring antigen)	complement fixation test	1:16
Listeria monocytogenes (heat inactivated antigen)	complement fixation test	1:16
Infectious bovine rhinotracheitis virus ("Los Angeles" strain)	serum neuralization test on Au-Bek cell line	1:4
Bovine viral diarrhea virus ("C24 Oregon" strain)	serum neutralization test on primary testicle cells	1:4
Parainfluenza-3 virus ("SF4" strain)	serum neutralization test on Au-Bek cell line	1:4
Bovine respiratory syncytial virus ("BOV X" strain)	serum neutralization test on Au-Bek cell line	1:4
Aujeszky's disease virus (natural outbreak strain)	serum neutralization test on IB-RS-2 cell line	1:4

[a] Following European Economic Community standard techniques for bovine brucellosis in wild boars and European Economic Community standard techniques for ovine brucellosis in fallow deer.

(National Institute of Wildlife Biology, Ozzano Emilia, Bologna, Italy). San Rossore Preserve is a roughly rectangular area enclosing about 50 km^2 bordering on the sea to the west, the Serchio River to the north, the Arno River to the South (43°47′ to 43°41′N and 10°16′ to 10°20′E). The preserve is fenced to the east. The preserve is one of the most "natural" localities in Italy. About 10 km^2 are cultivated land or fenced pastures, while the remaining 40 km^2 are mainly woodland available to wildlife. Seminatural grassland and coastal freshwater marshes are present also.

The preserve has the largest fallow deer population in Italy, with nearly 1,300 animals estimated in April 1984. The wild boar population also is extremely dense and has been estimated at 200 animals. Such populations still suffer from past improper management (mainly adult male shooting), which for many years caused overpopulation and a skewed sex and age structure. Management during the last 3 yr has attempted to correct this situation by a careful program of capture and shooting. Other wild mammals represented at San Rossore are red squirrels (*Sciurus vulgaris*), European rabbits (*Orictolagus cuniculus*), red foxes (*Vulpes vulpes*) and badgers (*Meles meles*).

About 200 holstein dairy cows are present in the preserve, but all are permanently housed. About 70 horses are raised for meat production in fenced pastures. No swine, sheep or goats are reared in the preserve; nevertheless, some contact between wild ungulates and sheep are likely to occur at the eastern border of the preserve.

Data collection

Blood samples were collected from the thoracic cavity of 43 fallow deer (22 males, 20 females, one animal in which the sex was not determined) and 20 wild boars (eight males, 12 females) shot at San Rossore Preserve during 1983 and 1984. Twenty-eight fallow deer and seven wild boar were >1 yr old. Twelve fallow deer and 18 wild boar sera were collected during the winter 1983; the remainder were collected during the winter 1984. Sera were separated by centrifugation and stored at −20 C until tested serologically.

Serology

Fallow deer sera were tested for antibodies against *B. abortus*, *L. monocytogenes*, *C. psittaci*, *C. burnetii*, IBR, PI-3, BVD and BRS viruses, while wild boar were tested with the same bacterial antigens plus Aujeszky's disease virus. Plate agglutination and complement fixation tests according to EEC standard techniques for laboratory diagnosis of brucellosis (Directive 64/432/EEC; Alton and Jones, 1967) were employed to detect *Brucella* sp. antibodies. The complement fixation test was employed for *C. psittaci*, *C. burnetii* and *L. monocytogenes* antibodies (Turilli et al., 1982, 1984). A serum neutralization test in microplates was used for

TABLE 2. Antibody prevalences and antibody titers in fallow deer in San Rossore, Italy.

Pathogen (test)	Results[a]	Prevalence	Geometric mean (minimum–maximum)
Brucella abortus (PAT)[b]	0/43	0%	3.16 (0–8 IU)
Brucella abortus (CFT)[c]	1/43	2%	2.82 (0–20 CFU)
Chlamydia psittaci (CFT)[c]	34/43	79%	20.70 (0–128)
Listeria monocytogenes (CFT)[c]	21/43	49%	7.47 (0–64)
Coxiella burnetii (CFT)[c]	3/43	7%	3.08 (0–16)
Infectious bovine rhinotracheitis virus (CFT)[c]	1/43	2%	2.97 (0–128)
Bovine viral diarrhea virus (SN)[d]	25/43	58%	4.93 (0–128)
Parainfluenza-3 virus (SN)[d]	11/43	26%	4.14 (0–128)
Bovine respiratory syncytial virus (SN)[d]	6/43	14%	3.48 (0–128)

[a] Number positive/number tested.
[b] Plate agglutination test.
[c] Complement fixation test.
[d] Serum neutralization test.

viral antibodies (Cancellotti et al., 1984; Cancellotti and Carlotto, 1987). Antigen strains, tests employed and threshold titers are reported in Table 1.

RESULTS

Antibody prevalences and geometric means of antibody titers are shown in Tables 2 and 3. In fallow deer the highest antibody prevalences were against *C. psittaci* (79%), BVD (or virus) (58%) and *L. monocytogenes* (49%). Prevalences of antibodies against two other pathogens were significant, namely PI-3 (26%) and BRS (or virus) (14%), while the remainder ranked very low (Table 2).

As far as wild boar are concerned, the only antibody positivity was against *Brucella* sp. antigen: four animals (20%) had complement-fixing antibodies (20 C.F.U.) but were negative with the plate agglutination test (Table 3). They were three females and one male, all <1 yr old.

In fallow deer there was only one statistically significant difference in antibody prevalence (Fischer's test; Siegel, 1956) based upon age, sex of animals or year of sampling. PI-3 was more prevalent in females ($P < 0.01$) and in 1984 than in the 1983 sampling period ($P < 0.05$; Table 4). Such results are consistent with comparisons among antibody titers (Student's t-test; Bailey, 1959). Indeed, the only significant difference in mean antibody titer was PI-3 which gave higher antibody titers in females than in males ($P < 0.01$) and in 1984 than in the 1983 sampling period ($P < 0.05$; Table 5).

TABLE 3. Antibody prevalences and antibody titers in wild boars in San Rossore, Italy.

Pathogen (test)	Results[a]	Prevalence	Geometric mean (minimum–maximum)
Brucella abortus (PAT)[b]	0/20	—	2.83 (0–4 IU)
Brucella abortus (CFT)[c]	4/20	20%	4.05 (0–20 CFU)
Chlamydia psittaci (CFT)[c]	0/20	—	0 (0–0)
Listeria monocytogenes (CFT)[c]	0/20	—	0 (0–0)
Coxiella burnetii (CFT)[c]	0/20	—	0 (0–0)
Aujeszky's disease virus (SN)[d]	0/20	—	0 (0–0)

[a] Number positive/number tested.
[b] Plate agglutination test.
[c] Complement fixation test.
[d] Serum neutralization test.

TABLE 4. Prevalences of various pathogens in fallow deer compared by means of Fisher's test.

Pathogen	Males[a]	Females[a]	Fisher's *P*
Chlamydia psittaci	17/22	16/20	0.565
Listeria monocytogenes	11/22	9/20	0.494
Bovine viral diarrhea virus	13/22	11/20	0.517
Parainfluenza-3 virus	1/22	10/20	0.001[c]
Bovine respiratory syncytial virus	2/22	4/20	0.286
Pathogen	**Juveniles[a]**	**Adults[a]**	**Fisher's *P***
Chlamydia psittaci	11/15	23/28	0.381
Listeria monocytogenes	9/15	12/28	0.226
Bovine viral diarrhea virus	9/15	16/28	0.564
Parainfluenza-3 virus	2/15	9/28	0.164
Bovine respiratory syncytial virus	2/15	4/28	0.656
	Year of sampling		
Pathogen	1983[a]	1984[a]	Fisher's *P*
Chlamydia psittaci	10/12	24/31	0.511
Listeria monocytogenes	5/12	16/31	0.511
Bovine viral diarrhea virus	9/12	16/31	0.147
Parainfluenza-3 virus	0/12	11/31	0.0145[c]
Bovine respiratory syncytial virus	0/12	6/31	0.121

[a] Number positive/number tested.
[b] $P < 0.05$.
[c] $P < 0.01$.

DISCUSSION

The presence of antibodies against *L. monocytogenes* in nearly one-half of the animals examined is analogous to the situation observed in other wild and domestic ungulates in Italy, and confirms that this infectious agent is prevalent and widespread (Corradini and Pecorari, 1981; Turilli et al., 1982; Sacco, 1985). Also, the prevalence of *C. burnetii* is analogous to that observed in other animal species (Moretti, 1984). The four wild boars and one fallow deer, which all showed a minimal reaction (20 C.F.U.) to the complement fixation test but were negative to the plate agglutination test for *B. abortus*, are interpreted as non-specific reactors.

As far as the other zoonotic agents are concerned, only the prevalence of antibodies against *L. monocytogenes* (49% in fallow deer) is noteworthy, while the prevalence of antibodies to the *C. burnetii* is very low (7% in fallow deer). *Chlamydia psittaci* strains of mammalian origin are of no known concern in public health (Buxton and Fraser, 1977; Eugster, 1980). The high prevalence of antibodies against *C. psittaci* and the respiratory disease complex viruses, suggest the need for more detailed investigations to determine their actual pathogenic role for wild ungulates.

In fallow deer, an increased and statistically significant ($P < 0.05$) prevalence of antibodies against PI-3 was observed between the 1983 and 1984 sampling periods which could suggest that the infection was introduced during that period. It must be stressed that 10 of 18 females and one of 13 males tested in 1984 proved positive for PI-3. It is interesting to note that the only positive male was a juvenile. This leads us to the hypothesis that the infection was introduced during the nonreproductive season, when females and juveniles are grouped in the central wooded part of the preserve where contacts with domestic ruminants are more likely. During this period males form even larger groups in the swamp along the sea side of the preserve, far from other contacts for the disease.

TABLE 5. Mean antibody titers in fallow deer compared by means of Student's *t*-test.

Pathogen	Males[a]	Females[a]	Student's *t*[b]
Chlamydia psittaci	19.46 (0–64)	20.95 (0–128)	0.2025 (40)
Listeria monocytogenes	7.48 (0–64)	7.15 (0–32)	0.1308 (40)
Bovine viral diarrhea virus	4.68 (0–16)	5.095 (0–128)	0.3355 (40)
Parainfluenza-3 virus	2.85 (0–8)	6.37 (0–128)	3.5175 (40)[d]
Bovine respiratory syncytial virus	2.999 (0–8)	4.153 (0–128)	1.4614 (40)
Pathogen	**Juveniles[a]**	**Adults[a]**	**Student's *t*[b]**
Chlamydia psittaci	15.83 (0–128)	23.90 (0–64)	1.1117 (41)
Listeria monocytogenes	9.04 (0–32)	6.74 (0–64)	0.8438 (41)
Bovine viral diarrhea virus	4.52 (0–8)	5.16 (0–128)	0.5099 (41)
Parainfluenza-3 virus	3.14 (0–8)	4.81 (0–128)	1.6408 (41)
Bovine respiratory syncytial virus	3.14 (0–8)	3.68 (0–128)	0.6814 (41)
	Year of sampling		
Pathogen	1983[a]	1984[a]	Student's *t*[b]
Chlamydia psittaci	25.23 (0–64)	19.18 (0–128)	0.6906 (41)
Listeria monocytogenes	5.69 (0–16)	8.299 (0–64)	1.0239 (41)
Bovine viral diarrhea virus	5.77 (0–32)	4.64 (0–128)	0.7893 (41)
Parainfluenza-3 virus	0.0 (0–0)	4.88 (0–128)	2.1667 (41)[c]
Bovine respiratory syncytial virus	0.0 (0–0)	3.83 (0–128)	1.4110 (41)

[a] Geometric mean (minimum–maximum).
[b] Degrees of freedom in parentheses.
[c] $P < 0.05$.
[d] $P < 0.01$.

In our investigation, a single fallow deer had an antibody titer of more than 1:128 against IBR, BVD, PI-3 and BRS viruses. Since IBR is an important disease for cattle in Italy and because breeding of wild ungulates for meat production is increasing as a form of exploitation of nonagricultural land, the epidemiology of IBR in deer is of particular interest. Although cervids are only secondary hosts for IBR virus and infection under natural conditions apparently is limited to serological positivity (Gibbs and Rweyemamu, 1977), disease following experimental infection and reisolation of IBR virus have been described (Chow and Davis, 1964). Therefore, the presence of single animals with high antibody titers could represent a source of concern when intensive breeding of wild ungulates for meat production is considered.

Our research emphasizes the need for permanent surveillance for zoonotic diseases on public lands. There are many important potential animal health implications related to the coexistence of wild ungulates with high densities of the human population and intensive domestic animal farming.

ACKNOWLEDGMENTS

We wish to express our appreciation to R. B. Griffith, A. Mantovani and S. Toso for their helpful advice and assistance during the preparation of this manuscript.

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Received for publication 4 March 1986.