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SEROEPIDEMIOLOGY OF CHLAMYDIAL INFECTIONS OF WILD RUMINANTS IN SPAIN

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ABSTRACT: Chlamydial infections were determined serologically among wild ruminants in the Nature Park of the Sierras de Cazorla, Segura y Las Villas (CNP; Spain). Sampling was done during the period from 1990–95. There were 1,244 blood samples collected, consisting of 490 from fallow deer (*Dama dama*), 343 from mouflon (*Ovis mussimon*), 283 from red deer (*Cervus elaphus*) and 128 from Spanish ibex (*Capra pyrenaica*). Specific complement-fixing antibodies of *Chlamydia* spp. were detected by means of microtechnique, using lipopolysaccharide antigen. The relationship of biological (species, sex, age), temporal (year) and territorial (central and peripheral areas) factors to seropositive prevalence was examined, and preliminary data were collected on whether or not sheep and goat herds grazing in the peripheral areas of the park also were infected with *Chlamydia* spp. Chlamydiosis was common in the four species of wild ruminants in the CNP in all the years studied. The prevalence of *Chlamydia* sp. in mouflon (37%) was significantly greater than in fallow deer (30%), and both had a significantly higher prevalence rate than Spanish ibex and red deer (both 24%). The four species of wild ruminants were similar in that they act as reservoirs of *Chlamydia* spp., although their receptivity may be different, and the infection can certainly be maintained among these animals by intra-group transmission. The differences in prevalences and geometric mean titers (GMT), both between the sexes (male versus female) and between different ages (adult versus juvenile), were insignificant in all four species. For all species of wild ruminants both prevalence rates and GMTs were greater in populations occupying the peripheral areas of the park than in those inhabiting the central area. Herds of sheep and goats had a high prevalence of chlamydiosis. Intertransmission of Chlamydia sp. between wild and domestic ruminants occurred through grazing on the same pastures. The highest mean prevalence (44%) of patent infections (CFT titers of $\geq$1:80) was detected in red deer, although this frequency was not significantly different from those observed in mouflon (39%), Spanish ibex (38%), and fallow deer (37%). The proportion of patent infection was higher in females than in males, and none of the juveniles (<2-yr-old) showed patent infections. The prevalence of predicted patent chlamydial infections was always higher in the peripheral areas of the park, although only among mouflon and fallow deer were the differences statistically significant.

Key words: *Chlamydia* spp., complement fixation test, fallow deer, mouflon, red deer, seroepidemiological study, Spain, Spanish ibex, wild ruminants.

INTRODUCTION

The genus *Chlamydia* currently comprises four species consisting of *C. trachomatis* (Wang and Grayston, 1991), *C. psittaci* (Page, 1968), *C. pneumoniae* (Grayston et al., 1989) and *C. pecorum* (Fukushi and Hiray, 1993). All these species present various common antigens, the most important of which is the lipopolysaccharide antigen (LPS), which can easily be demonstrated by complement fixation (Brade et al., 1986). The LPS antigen presents a specific epitope of the genus *Chlamydia* (Caldwell and Hitchcock, 1984), but it can react unspecifically with rough mutants of *Salmonella typhimurium* and *S. minnesota* (Nurminen et al., 1984).

Chlamydia psittaci is very heterogeneous both genetically and immunologically. It infects a wide variety of hosts, although a certain association exists among a range of hosts and serotype (Andersen, 1991). The strains of *C. psittaci* isolated from ruminants can be catalogued into two serotypes (Schachter et al., 1974). Those belonging to serotype 1, which constitute an antigenically homogeneous group, are very virulent strains (Rodolakis and Sour-

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iau, 1992); conversely, serotype 2 consists of a group of low-virulence and immunologically heterogeneous strains (Rodolakis et al., 1989). Recently, from those low-virulence strains isolated from ruminants and pigs, belonging mostly to *C. psittaci* serotype 2, the species *C. pecorum* has been created (Fukushi and Hirai, 1992). But no antigenic relationship has been found between these *C. pecorum* serotype 2 strains and the ruminant serotype 1 strains of *C. psittaci* (Salinas et al., 1996).

All chlamydial species are pathogenic in animals, including *C. pneumoniae* in koalas (Storz and Kaltenboeck, 1993) and horses (Storey et al., 1993), and *C. trachomatis* is widespread in swine (Schiller et al., 1997). In ruminant species *C. psittaci* is an important pathogen. It mainly induces abortion and genital infections, and can also cause enteritis, conjunctivitis, mastitis, pneumonia polyarthritis and meningoencephalitis (Salinas et al., 1995). Alternatively, *C. pecorum* usually generates infections which are not apparent, although in some cases it can develop enteritis, polyarthritis and meningoencephalitis, but without severe clinical signs (Fukushi and Hirai, 1993).

Despite the fact that chlamydiosis has been the main cause of abortion in small ruminants in Spain (Gil and Blasco, 1993), few serological studies have been conducted to determine the seroprevalence to *Chlamydia* spp. in these animals. Using the complement fixation test (1:20 cut-off titers), the rates of seroprevalence to *Chlamydia* spp. reported in sheep from different Spanish regions were as follows: 56% in Zaragoza (Pérez et al., 1994), 51% in Madrid (Mainar-jaime et al., 1998), 28% in Córdoba (Cuello, 1979) and 25% in Murcia (Cuello et al., 1992), and 14% in goats in Murcia (Cuello et al., 1992). Furthermore, in some populations of wild ruminants in Andalusia (Spain), serological surveys indicate that chlamydial infections also are frequent in both red deer (12%) and roe deer (16%) (León-Vizcaíno et al., 1994 a, b).

The "Sierras de Cazorla, Segura and Las Villas" Nature Park (CNP) (59°54′N, 17°51′E) is a hilly area of 2,140 km^2 located at the eastern end of the Betic Mountain (Spain). It has sizeable populations (Fandos et al., 1991) of red deer (*Cervus elaphus*), mouflon (*Ovis mussimon*), fallow deer (*Dama dama*) and Spanish ibex (*Capra pyrenaica*), which are hunted. However, due to a serious epidemic of scabies (*Sarcoptes scabiei*) in 1987 (León-Vizcaíno et al., 1992a) the hunting of the Spanish ibex was discontinued until the 1996–97 season. In a wide peripheral strip, at the border of the park, wild ruminants share their habitat with numerous herds of sheep and goats.

The purposes of this study were firstly to determine the prevalence of *Chlamydia* spp. antibodies over a long period (1990–95) in the four species of wild ruminants living in the CNP, and secondly to examine the relationship of biological factors (species, sex, age), temporal factors (year) and territorial factors (central and peripheral areas) to seropositive prevalence. Studies were initiated to find out whether or not herds of sheep and goats grazing in the peripheral area of the park also were infected with *Chlamydia* spp. Chlamydiosis was identified by determining complement-fixing antibodies. While this is only an initial approach to monitoring the disease, it has the advantage of detecting both present and past experience with these infections.

MATERIALS AND METHODS

The prevalence of chlamydiosis was investigated during the 6 yr period from 1990–95 in wild ruminants from the CNP. The population size, as estimated by Fandos et al. (1991), was 3,960 red deer, 4,680 fallow deer, 3,240 mouflon, and 475 Spanish ibex. Ruling out those samples that showed anticomplementary activity, we were left with only 833 (67%) of the original 1,244 sera that could be used for immunological study, comprising 306 from fallow deer, 239 from mouflon, 234 from red deer, and 54 from Spanish ibex.

Blood samples were collected by extracting blood clots from the cardiac cavities of hunted

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animals or by puncturing the jugular veins of captured animals under anaesthesia by telinject darts with a combination of 10 μg/kg body weight of Ketamine (Imalgene®, Rhône Mérieux, Lyon, France) and 200 μg/kg body weight of Xylacine (Rompum®, Bayer, Leverkusen, Germany). Sampling was done at random, using animals which had been hunted or selectively killed by forest guards after showing weakness or suspected disease, or as a means of controlling the respective populations of the wild ruminants.

A microtechnique complement fixation test (CFT) for chlamydial antibodies was performed as described by Giauffret and Russo (1976). The antigen used was lipopolysaccharide (LPS), which is common to all the members of the genus *Chlamydia,* is thermostable, and can be extracted from the cell wall with ether (Volkert and Christensen, 1955). This was kindly provided by J. Salinas (Microbiology and Immunology, Department of Animal Pathology, Murcia University, Murcia, Spain). Two-fold dilutions of sera (1:10 to 1:320) were tested and the titer defined as the reciprocal of the highest dilution showing a positive reaction. A cut-off titer of 1:20 was selected because it is considered to be indicative of chlamydial infection (Cuello et al., 1992). Sera with anti-complementary effects were judged as uninterpretable. The titers of CFT antibodies of seropositive animals were summarized as the mean value of the geometric mean titer (GMT) (Thrusfield, 1990).

As far as the stage of infection was concerned, the indications of Giauffret and Russo (1976) and Rodolakis and Russo (1984) were followed. These authors reported that titers of ≤1:40 were indicative of latent or chronic infections, and titers of ≥1:80 indicated patent or active infections.

In order to analyze both the natural nidality of *Chlamydia* spp. in the wild ruminant and the potential contact with small domestic ruminants, the animals were divided into two territorial groups, according to whether they had been captured in the central or peripheral area of the CNP (Fig. 1). There is no formal obstruction preventing the animals' movements between the two areas. However, the rugged topography constitutes a boundary for the regular movements of wild animals, especially females. The separation between the area of intensive hunting and the rest of the park is rather poorly defined; it is determined by farming as opposed to stockbreeding areas. The wild ruminants were sub-classified into juveniles and adults, and males and females.

The situation regarding chlamydiosis was investigated in the 60 small domestic ruminant herds (52 mixed but consisting mainly of sheep, 3 of sheep, and 5 of goats) which graze in the peripheral areas of the park. 33% of the herds (20/60) had some past record. Diagnostic methods for the detection of *Chlamydia* spp. include the Stamp staining technique (Schachter and Dawson, 1979) and the detection of the chlamydial group antigen by immunoassays (IDEAI™ PCE Chlamydia®, DAKO Diagnostic Ltd., UK) from the swab of vaginal fluid. All the herds (in a statistically significant sample) were subjected to immunological analysis by CFT in order to find out whether they included infected animals.

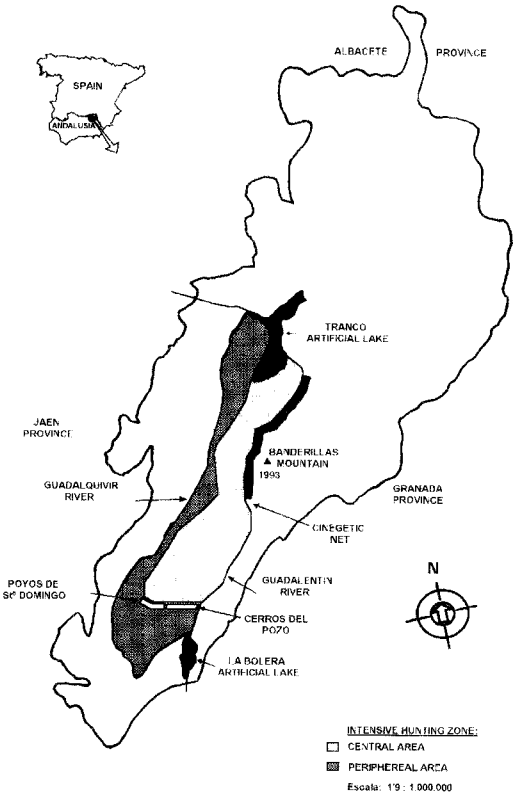


FIGURE 1. Map of Sierras de Cazorla, Segura y Las Villas Nature Park, Jaen, Spain.

Statistical analysis was performed with the Epiinfo 6 integrated epidemiological statistics package (Dean et al., 1994) and SPSS software (Ferrán, 1996). Differences among prevalence rates of seropositives in relation to the four host species, the two areas and the 6 yr were analyzed using a Yates-corrected Chi-square test. Differences between geometric mean titers (GMT) for each species, area, year and patent infection were analyzed using Fisher's exact test. The correlation of seropositives and patent infections between species was evaluated using

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TABLE 1. Prevalence of seropositives (titer ≥1:20 for complement fixation test) by sex, age and year (1990–95) for *Chlamydia* spp. in wild ruminants from Sierras de Cazorla, Segura y Las Villas Nature Park (Spain).

Species/category	1990	1991	1992	1993	1994	1995	1990–95 period
Mouflon	8/22 (37)[a]	—	12/31 (39)	18/45 (40)	25/72 (35)	26/69 (38)	89/239 (37)[e]
							37.8 ± 0.2[b]
Females	6/18 (33)	—	10/27 (37)	17/43 (40)	24/69 (35)	25/66 (38)	83/223 (37)
Males	1/2 (50)	—	1/2 (50)	1/2 (50)	1/2 (50)	1/2 (50)	4/10 (40)
Juveniles	1/2 (50)	—	1/2 (50)	—	0/1 (0)	0/1 (0)	2/6 (33)
Fallow deer	2/9 (22)	29/96 (30)	10/54 (19)[d]	3/10 (30)	27/72 (38)[e]	20/65 (31)	91/306 (30)
							28.3 ± 2.2[b]
Females	1/6 (17)	26/88 (30)	9/49 (18)	3/9 (33)	25/65 (38)	18/58 (31)	82/275 (30)
Males	1/2 (50)	2/6 (33)	1/3 (33)	0/1 (0)	1/4 (25)	1/4 (25)	6/20 (30)
Juveniles	0/1 (0)	1/2 (50)	0/2 (0)	—	1/3 (33)	1/3 (33)	3/11 (27)
Red deer	13/33 (39)[c]	7/26 (27)	4/19 (21)	12/40 (30)	15/66 (23)	6/50 (12)[d]	57/234 (24)[f]
							25.3 ± 1.1[b]
Females	10/27 (37)	7/24 (29)	2/10 (20)	10/32 (31)	13/56 (23)	5/42 (12)	47/191 (25)
Males	2/4 (50)	0/1 (0)	2/8 (25)	1/5 (20)	1/4 (25)	0/2 (0)	6/24 (25)
Juveniles	1/2 (50)	0/1 (0)	0/1 (0)	1/3 (33)	1/6 (17)	1/6 (17)	4/19 (21)
Spanish ibex	7/25 (28)	5/26 (19)	—	—	—	1/3 (33)	13/54 (24)[f]
							23.3 ± 3.0[b]
Females	3/10 (30)	3/14 (21)	—	—	—	1/3 (33)	7/27 (26)
Males	3/13 (23)	2/12 (17)	—	—	—	—	5/25 (20)
Juveniles	1/2 (50)	—	—	—	—	—	1/2 (50)

[a] Number positive/number tested without anti-complementary activity (% positive).
[b] CI_{95} = Confidence interval 95%.
[c,d] Group of values, for each species, with interannual significant differences (a versus b, $P < 0.05$).
[e,f] Group of values with interspecific significant differences (c versus d, $P < 0.05$).

Pearson's linear correlation (Fleiss, 1981). The level of significance was set at $P \leq 0.05$.

RESULTS

Chlamydial infection was highly prevalent in the four species of wild ruminants in the CNP in all the years studied (Table 1). There were seropositive animal prevalence rates (CI_{95} = mean ± 2 sample estimated standard error) of 37 ± 2% in mouflon, 28 ± 5% in fallow deer, 27 ± 8% in Spanish ibex, and 25 ± 6% in red deer. Some species were more infected than others; mouflon had significantly greater prevalence rates than red deer and Spanish ibex. Fallow deer showed an intermediate level of prevalence, not significantly different from the other species (Table 1).

In relation to the collective intensity of the immunological response (geometrical mean titer, GMT) of anti-*Chlamydia* spp. fixing complement antibodies calculated throughout the sampling period, the values for GMT (Table 2) in Spanish ibex (1:55) and red deer (1:54) also were similar, but, unlike the respective prevalence rates, were greater than in mouflon (1:49) and fallow deer (1:48). The difference between these two pairs of host species was statistically significant.

Using the hypothesis that an indirect criterion for establishing whether chlamydiosis is present in active course (patent infection) is to detect anti-*Chlamydia* spp. fixing complement antibody titers equal to or above 1:80, high rates of prevalence of this immunological reaction were recorded in every species every year (Table 3). The overall specific proportion of patent chlamydiosis infection was only slightly different from species to species, ranging from 44% in red deer to 39% in mouflon, 38% in Spanish ibex and 37% in fallow deer. These differences are not statistically significant. None of the 38 juvenile animals (<2-yr-old) showed patent infections. The proportion of patent infection was system-

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TABLE 2. Geometric mean titer of *Chlamydia* spp. complement fixing antibodies in wild ruminants across year and regions (central and peripheral areas) from Sierras de Cazorla, Segura y Las Villas Nature Park (Spain) during 1990–95.

Species	1990	1991	1992	1993	1994	1995	1990–95
Mouflon	61.7[a]	—	50.4[b]	52.4[b]	50.0[b]	42.2[c]	49.0[e]
Fallow deer	40.0[c]	46.2[b]	42.9[b]	40.0[c]	50.4[b]	56.6[a]	48.8[e]
Red deer	58.1[b]	44.2[c]	80.0[a]	47.6[b]	45.9[c]	89.8[a]	54.2[d]
Spanish ibex	53.8[b]	60.6[a]	—	—	—	40.0[c]	55.1[d]
			Central Area			Peripheral area	
Mouflon			43.1			56.1[e]	
Fallow deer			43.3			62.0[d]	
Red deer			43.6			56.2[e]	
Spanish ibex			40.0			60.6[d]	

[a,b,c] Group of values, for each species, with inter-annual significant differences (a versus b versus c, $P < 0.05$).
[d,e] Group of values with inter-specific significant differences (d versus e, $P < 0.05$).

atically higher in females than in males (Table 3); 1.6 times higher among mouflon, and more than twice as high in fallow deer (2.3 times), Spanish ibex (2.8 times) and red deer (3 times). The difference between the sexes was significant (Fisher's exact one-tail test; $P = 0.07$) among red deer but not in the other species ($P > 0.20$).

Due both to the non-existence of Spanish ibex samples in three of the years (1992, 1993, 1994) and to the very low sample size for this species in 1995 we did not attempt to evaluate year to year differences statistically. Analysis of the chronological patterns of annual prevalence rates for all species in the six-year period from 1990 to 1995 (Table 1) revealed no significant differences. There were, however, some statistically significant waves of

TABLE 3. Proportion of active seroreactors for *Chlamydia* spp. (titer ≥1:80 for complement-fixation test) by sex, age and year (1990–95 yr) in wild ruminants from Sierras de Cazorla, Segura y Las Villas Nature Park (Spain).

Species category	1990	1991	1992	1993	1994	1995	1990–95
Mouflon	4/8 (50)[a]	—	5/12 (42)	9/18 (50)	10/25 (40)	7/26 (27)	35/89 (39)
Females	4/6 (67)	—	5/10 (50)	8/17 (47)	10/24 (42)	7/25 (28)	34/83 (41)
Males	0/1 (0)	—	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	1/4 (25)
Juveniles	0/1 (0)	—	0/1 (0)	—	—	—	0/2 (0)
Fallow deer	0/2 (0)	10/29 (34)	3/10 (30)	1/3 (33)	11/27 (41)	9/20 (45)	34/91 (37)
Females	0/1 (0)	10/26 (38)	3/9 (33)	1/3 (33)	10/25 (40)	9/18 (50)	32/82 (39)
Males	0/1 (0)	0/2 (0)	0/1 (0)	—	1/1 (100)	0/1 (0)	1/6 (17)
Juveniles	—	0/1 (0)	—	—	0/1 (0)	0/1 (0)	0/3 (0)
Red deer	6/13 (46)	3/7 (43)	2/4 (50)	5/12 (42)	5/15 (33)	4/6 (67)	25/57 (44)
Females	5/10 (50)	3/7 (43)	2/2 (100)	5/10 (50)	5/13 (38)	4/5 (80)	24/47 (51)
Males	1/2 (50)	—	0/2 (0)	0/1 (0)	0/1 (0)	—	1/6 (17)
Juveniles	0/1 (0)	—	—	0/1 (0)	0/1 (0)	0/1 (0)	0/4 (0)
Spanish ibex	3/7 (43)	2/5 (40)	—	—	—	0/1 (0)	5/13 (38)
Females	2/3 (67)	2/3 (67)	—	—	—	0/1 (0)	4/7 (57)
Males	1/3 (33)	0/2 (0)	—	—	—	—	1/5 (20)
Juveniles	0/1 (0)	—	—	—	—	—	0/1 (0)

[a] Number of animals with active (patent) infection (titer ≥1:80 CFT antibody)/number of seroreactors (titer ≥1:20 CFT antibody) (% animals with patent infection).

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TABLE 4. Prevalence of chlamydiosis in species found in the central and peripheral area of Sierras de Cazorla, Segura y Las Villas Nature Park (Spain) during the period 1990–95.

Species/ category	Central area	Peripheral area
Mouflon	46/140 (33)[a,b]	43/99 (43)
Females	4/133 (33)	39/90 (43)
Males	2/7 (29)	1/3 (33)
Juveniles	—	3/6 (50)
Fallow deer*	61/238 (26)	30/68 (44)
Females	55/212 (26)	28/63 (44)
Males	4/15 (27)	2/5 (40)
Juveniles	2/11 (18)	30/68 (44)
Red deer*	8/48 (17)[c]	49/186 (26)
Females	6/40 (15)	40/151 (26)
Males	1/4 (25)	5/20 (25)
Juveniles	1/4 (25)	4/15 (27)
Spanish ibex	3/26 (12)[c]	10/28 (36)
Females	1/10 (10)	6/17 (35)
Males	2/14 (14)	4/11 (36)
Juveniles	0/2 (0)	—

[a] Number positive/number tested without anti-complementary activity (% positive).

[b,c] Group of values with inter-specific significant differences in each area (a versus b, $P < 0.05$).

* Group of values with interterritory significant differences ($P < 0.05$) in respective species.

TABLE 5. Proportion of predicted chlamydial patent infections by serology in wild ruminants from the central and peripheral areas of the Sierra de Cazorla, Segura y Las Villas Nature Park (Spain), 1990–95.

Species/category	Central area	Peripheral area
Mouflon*	12/46 (26)[a]	23/43 (53)
Females	12/44 (27)	22/39 (56)
Males	0/2 (0)	1/1 (100)
Juveniles	—	0/3 (0)
Fallow deer*	18/61 (30)	16/30 (53)
Females	18/55 (31)	15/28 (54)
Males	0/4 (0)	1/2 (50)
Juveniles	0/2 (0)	—
Red deer	2/8 (25)	23/49 (47)
Females	2/6 (33)	22/40 (55)
Males	0/1 (0)	1/5 (20)
Juveniles	0/1 (0)	0/4 (0)
Spanish ibex	1/3 (33)	4/10 (40)
Females	1/1 (100)	3/6 (50)
Males	0/2 (0)	1/4 (25)
Juveniles	—	—

[a] Number of animals with patent infection (titer ≥ 1:80)/ number of seropositive animals (titer ≥ 1:20) (% animals with patent infections)

* Significant difference ($P < 0.05$) between study areas.

epidemic both in fallow deer with the highest rate of 38% (1994) and lowest rate of 22% (1992), and in red deer with the highest rate of 39% (1990) and lowest rate of 12% (1995). Alternatively, the chronological evolution of anti-*Chlamydia* spp. antibodies (Table 2) showed very irregular profiles of GMT, whose high, middle, and low levels were significantly different. However, only in red deer was a definite pattern of evolution present, reaching peaks of up to 1:80 in 1992 and 1995. In addition, none of the annual GMT profiles corresponding to the four host species were parallel. In none of these species was any statistical association detected by correlation or regression analysis between the annual profiles of prevalence and of GMT. Nor were the differences of prevalence rates between sexes (male versus female) and ages (adult versus juvenile) significant in any of the species (Table 1).

For each species of wild ruminants both frequencies of occurrence of antibodies and GMT were greater in the populations occupying the peripheral area of the park than in those inhabiting the central area (Tables 2, 4). Within the peripheral area all four species were highly infected by *Chlamydia* spp. Fallow deer (44%) and mouflon (43%) showed higher prevalence rates than red deer (26%), which also were significantly different from Spanish ibex (36%). In the central area chlamydial infection also affected these species; prevalence rates were 33% for mouflon 26% for fallow deer, 17% for red deer, and 12% for Spanish ibex. However, there were no significant differences except between mouflon and Spanish ibex.

The prevalence of predicted patent chlamydial infections was always higher in the peripheral area (Table 5); although only among mouflon ($P = 0.01$) and fallow deer ($P = 0.02$) was the difference between the proportions of patent chlamydiosis from peripheral and central areas statistically significant. Nevertheless, the risk (odds ratios very close to 1.0) of find-

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TABLE 6. Estimation of chlamydial infection statistical risk for each wild ruminant specie from Sierras de Cazorla, Segura y Las Villas Nature Park (Spain), during 1990–95.

Species	Cases[a]	Control[b]	χ^2	*P*	O.R.[c]	CI_{95}[d]	
Mouflon	89	150	7.86	0.005	1.60	1.15	2.22
Fallow deer	91	215	0.00	0.957	0.98	0.71	1.35
Red deer	57	177	4.58	0.03	0.68	0.47	0.97
Spanish ibex	13	41	0.69	0.40	0.73	0.36	0.43

[a] Case (seropositive).
[b] Control (seronegative).
[c] O.R. = Odds ratio.
[d] CI_{95} = Binomial exact confidence interval 95%.

ing active infections due to *Chlamydia* spp. (Table 6) appeared to be independent of area in all species. This is due to the contribution of females, since juvenile and males did not present patent infection in the central area. In the peripheral area the prevalence of patent infections in females was high and similar for the four species of wild ruminants. While in males the prevalence of patent infections was higher in mouflon and fallow deer than in Spanish ibex and red deer (Table 5), although the respective sample sizes were very scanty.

Of the 60 small domestic ruminant herds grazing in the peripheral areas of the park, 27 (45%) included animals which were chlamydial antibody carriers (Table 7). In the infected herds, the mean chlamydial infection prevalence rate was 32% (CI_{95} = 31.8 ± 8.8). The intensity of infection as measured by titers (GMT) was 1:128, and in most cases (78%) chlamydiosis in infected herds of sheep and goats was manifested as a patent infection. At the time of the serological survey the current abortion rate resulting from the chlamydia was (3/60) 5%.

DISCUSSION

Infections by *C. psittaci* and *C. pecorum* in these ruminants in this park in Spain are among the most prevalent in wild ruminants from Europe. However, it seems that pathogen activity does not contribute with the same frequency to the causality of cases of disease in these animals (Bourgogne, 1990; Cordier, 1990). The pathological impact on wild populations has been proved when keratoconjunctivitis is in evidence (Oudar et al., 1985; Tournut et al., 1985; Sánchez Belda and Martínez Ferrando, 1985). This, though, is not the case with abortions, due to the practical impossibility of finding fetuses and placenta in the mountains before they are consumed by carnivores; this leaves us with only one approach, namely the chronological comparison of the birth rates and their correlation with the seroprevalence rates and the mean antibody titers. Furthermore, serological analysis is the only way of specifying the reservoir role of these species (Bourgogne, 1990).

The complement fixation test (CFT) is the most commonly used and widely acepted serological test for diagnosing chlamydiosis (Office International des Epizooties, 1992), using as an antigen lipopolysaccharide from the chlamydial cell wall (Galanos et al., 1969). Chlamydial CFT is a method which offers the advantage of being usable in several animal species. It is very insensitive in large ruminants (cattle) but less so in small ruminants (Kaltenboeck et al., 1997; Sting, 1997; Perez-Martínez et al., 1986). It also has some disadvantages: the existence of sera with anticomplementary activity or haemolytic sera, which are very common in the conditions under which samples are obtained from wild populations, gives rise to non-interpretable sera; for this reason these sera are not valid research material for mass immunological analysis. Another

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TABLE 7. Prevalence of seropositives, geometric mean titer (GMT) and frequency of patent infections in herds of small domestic ruminants grazing in the peripheral area of the Sierra de Cazorla, Segura y Las Villas Nature Park (Spain) infected by *Chlamydia* spp.

Herd seropositive	Animals	Infected/analyzed	GMT	Patent infection/infected
1	256	4/13 (31)	47.6	2/4 (50)
2	348	4/13 (31)	56.6	2/4 (50)
3	490	1/24 (4)	80.0	1/1 (100)
4	160	2/9 (22)	40.0	0/2
5	660	2/29 (7)	56.6	1/2 (50)
6	197	5/11 (45)	105.5	4/5 (80)
7	477	8/22 (36)	113.1	6/8 (75)
8	230	7/12 (58)	97.5	5/7 (71)
9	178	2/10 (20)	226.2	2/2 (100)
10	142	3/9 (33)	59.6	1/3 (33)
11	380	3/20 (15)	63.5	2/3 (67)
12	45	2/4 (50)	80.0	2/2 (100)
13	105	3/7 (43)	160.0	3/3 (100)
14	130	6/8 (75)	160.0	6/6 (100)
15	188	6/11 (54)	63.5	3/5 (50)
16	920	19/52 (36)	107.1	14/19 (74)
17	104	1/7 (14)	40.0	0/1
18	712	30/31 (97)	60.6	15/30 (50)
19	450	8/21 (38)	146.7	8/8 (100)
20	303	5/16 (31)	69.6	4/5 (80)
21	480	2/22 (9)	40.0	0/1
22	746	8/34 (23)	113.1	8/8 (100)
23	591	1/27 (4)	40.0	0/1
24	206	1/12 (8)	80.0	1/1 (100)
25	355	2/17 (12)	40.0	0/1
26	125	4/7 (57)	160.0	4/4 (100)
27	226	4/12 (8)	40.0	0/1
Total	9.189	143/460 (31.8 ± 8.8)[a]	128.3 ± 57.6[a]	94/135 (77.6 ± 10)[a]

[a] CI_{95} = Confidence interval 95%.

serious disadvantage is the difficulty in detecting specific antibodies below genus level (Rodolakis, 1988), and also there are serious problems of sensitivity when working with animals with low titers (Markey et al., 1993).

The high prevalence of chlamydial infection observed in the wild ruminants (mouflon, fallow deer, Spanish ibex and red deer) of the CNP showed that the four species act similarly as reservoirs of *Chlamydia* spp., although their receptivity may be different. So, the infection can certainly be maintained among these animals by intra-group transmission because these species represented a population continuum (Thrusfield, 1990) with considerable contact and interchange (Escos and Alados, 1988). Immunological surveys seem to suggest that *Chlamydia* spp. are widely disseminated into wild ruminant populations from Andalusia: in Spanish ibex (León-Vizcaíno et al., 1992b), in roe deer (León-Vizcaíno et al., 1994a), and in red deer (León-Vizcaíno et al., 1994a, b).

Serological studies of the prevalence of chlamydiosis in wild ruminants offer very varied results according to their location. In general, in Italy and in Spain there is a higher rate of prevalence than in Germany and in France. The prevalence of mouflon seropositives to chlamydial infections detected in CNP (37%) is higher than those observed in France during 1980–89, which were 7% (4/58) (Cordier, 1991), falling to 3% (1/31) during the 1990–92 period

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(Gourreau et al., 1993), and in Germany from 1984 to 1989 the two animals analyzed were seronegative (Dedek et al., 1991). In Italy, the only animal investigated was seropositive (Andreani et al., 1986).

Among the fallow deer population from CNP the prevalence rate (30%) is higher than the 9% reported in Germany from 1984 to 1989 (1/11) (Dedek et al., 1991). In Italy four fallow deer analyzed during 1980–81 were seropositive (Corrandini and Pecorari, 1981), although seven animals investigated during 1981–84 were seronegative (Andreani et al., 1986).

The prevalence of chlamydiosis we noted in Spanish ibex (24%) lies within the range reported in various surveys conducted on ibex (*Capra ibex*) populations in France. Various studies obtained positive results, with prevalences ranging from 13% to 30% with actual values of 11/83 (Baradel et al., 1990), 19/124 (Bourgogne, 1990), 27/161 (Cordier, 1991), and 3/10 (Gauthier, 1994). It also was higher than the 12% (3/25) detected in Spanish ibex males captured during 1991–92 in Sierra de Las Nieves Nature Park (Málaga, Spain) (León-Vizcaíno et al., 1992b).

The high chlamydiosis prevalence rate among red deer (24%) is surpassed by the prevalence detected in Italy where there was 100% (23/23) during 1980–81 (Corrandini and Pecorari, 1981) and 59% (26/44) during 1981–84 (Andreani et al., 1986). Conversely, it is higher than that observed in the area of the Alps and Haute-Marne (France) from 1987–92 which ranged from 4% to 10% with actual values of 5/52 (Peyre-Mandras, 1990), 2/46 (Barrat, 1992), 3/64 (Gourreau et al., 1993), and in Germany where during 1984–89 a rate of 5% was found (18/368) (Dedek et al., 1991). Anti-*Chlamydia* antibodies were not found at all in 500 red deer sampled in New Caledonia from 1980–90 (Desvals et al., 1991).

In sheep, complement-fixing antibodies form within 7 to 10 days after the beginning of active infection and may persist for 30 mo (Jensen and Swift, 1982). Therefore, high positive titers indicate a recent episode of chlamydial infection, be it clinically apparent or (more often) not. Infected ewes generally have low or moderate levels of complement-fixing antibodies but aborting ewes experience an episode of chlamydaemia which often results in a significant post-abortion rise in antibody titer (Aitaken, 1991). In the four species of wild ruminants in the CNP, throughout the whole period of this study, the geometric mean CF antibody titer was ≥1:40. The exceptionally high GMT results (≥1:80) in female fallow deer in 1992 and 1995 could be related to clinical episodes.

For each species of wild ruminants both prevalences of antibodies and GMT were greater in populations occupying the peripheral area of the park than in those inhabiting the central area. In CNP not only are there various herds of sheep and goats grazing; but in addition, in these herds chlamydiosis has been shown to be a frequent infection (45% of the herds and 32% of the seropositive animals), which has been rigorously detected in patent form (global GMT 1:128, 78% of the cases with a GMT of >1:80). In addition it has been shown that it is a frequent cause of episodes of abortion (at the time of the survey the current abortion rate on account of chlamydia was 5%). Due to all the above it is easy to deduce that the transmission of chlamydia between wild and domestic ruminants is probable, as Cordier (1990) and Hars (1992) have already suggested by correlating a high level of chlamydiosis in wild ruminants in Savoie (France) with a serious epizootia suffered by ovine farming herds. In the same way, other authors state that the inter-transmission of chlamydial infection between wild and domestic ungulates occurred by grazing on the same pastures (Andreani et al., 1986; Gourreau et al., 1993; Hars, 1992), despite the low resistance level of the germ in the external environment and the low lambing and kidding rates in the period when the pasture is shared (Bourgogne, 1990).

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There are few serological surveys of chlamydiosis in wild ruminants, and none of them has analyzed immunological aspects such as the prevalence of patent infections according to the complement fixation antibody titer (≥1:80) (Rodolakis and Russo, 1984) or the geometric mean titer of the seropositive population detected. For this reason, many of our immunological observations cannot be compared with previous studies. Only Cordier (1990) looked at these issues. This author detected that in 75% of ibex and mouflons from Savoie (France) the chlamydial infection was in latent state (CFT ≤ 1:40), and sees this as the result of an infection passed on through *Chlamydia* spp. or through a less intensive immune response to the infection in wild animals than in domestic animals. In the wild ruminants in the CNP latent chlamydial infections were marginally less frequent at 56% in red deer, 61% in mouflon, 62% in Spanish ibex, and 63% in fallow deer.

During the study period no significant differences in the mean prevalence of patent infection were observed among the four species of wild ruminants in the CNP. No patent infections were observed in juveniles. In males patent infections were detected during the 6 yr period in all species, in the years when the highest infection prevalence rates occurred. While chlamydial infection may have been nonselective for sex or age, patent infection apparently selected disproportionately against females.

The patent chlamydial infection prevalence rates were significantly higher in mouflons and fallow deer inhabiting the peripheral area than in the central area, while in red deer and Spanish ibex no significant difference was observed. This might be explained by the fact that the mouflon is an animal which lives in closely-knit groups with the herds of sheep, even during the day, and fallow deer use the pasture left by domesticated animals during the night; while red deer and Spanish ibex keep away from the land grazed by domesticated animals. Additionally, episodes of patent infections are typically precipitated by periods of stress, such as scarcity and imbalance of food sources, water, etc. All this seems to indicate that the progressive invasion of domestic livestock is causing a centripetal spread of chlamydiosis from the peripheral area, where domestic and wild ruminants cohabit and where the probability of infection is greater.

Health work should be orientated toward practices which prevent the penetration and colonization of pathogenic agents, and the prevention of factors that weaken the animal and make it more sensitive. Some possible strategies are (1) to adjust the animal load to the nutritional offer of the territory and to supplement food in times of special need; (2) to regulate the population by selectively hunting weak or infirm animals throughout the year; (3) a health watch, with a study of infection and parasitosis in animals hunted cynegetically or selectively and in those captured for any reason; and (4) health control of domestic livestock (regular deparasitization, mass immunization against the main exogenous and endogenous diseases, early diagnosis, and treatment and/or sacrifice of diseased animals) cohabiting with wild species (León-Vizcaíno et al., 1994a).

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