EPIDEMIOLOGY OF MYCOPLASMA AGALACTIAE INFECTION IN FREE-RANGING SPANISH IBEX (CAPRA PYRENAICA) IN ANDALUSIA, SOUTHERN SPAIN

Authors: Verbisck-Bucker, G., González-Candela, M., Galián, J., Cubero-Pablo, M. J., Martín-Atance, P., et al.

Source: Journal of Wildlife Diseases, 44(2) : 369-380

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.2.369

Journal of Wildlife Diseases, 44(2), 2008, pp. 369–380

EPIDEMIOLOGY OF *MYCOPLASMA AGALACTIAE* INFECTION IN FREE-RANGING SPANISH IBEX (*CAPRA PYRENAICA*) IN ANDALUSIA, SOUTHERN SPAIN

G. Verbisck-Bucker,[1] M. González-Candela,[1,3] J. Galián,[2] M. J. Cubero-Pablo,[1] P. Martín-Atance,[1] and L. León-Vizcaíno[1]

[1] Infectious Diseases, Department of Animal Health, Faculty of Veterinary Medicine, University of Murcia, Campus Universitario de Espinardo, 30100 Murcia, Spain
[2] Animal Biology, Department of Zoology and Physical Anthropology, Faculty of Veterinary Medicine, University of Murcia, Campus Universitario de Espinardo, 30100 Murcia, Spain
[3] Corresponding author (email: monica@um.es)

ABSTRACT: *Mycoplasma agalactiae* is the main causal agent of contagious agalactia syndrome in Spain. It is a severe disease of small ruminants, endemic in Mediterranean countries, that is characterized by mastitis, arthritis, and keratoconjunctivitis. This paper investigates the temporal, spatial, and host-related factors in the distribution of *M. agalactiae* infection from October 1996 to November 1998 and March 2002 to May 2003 in Spanish ibex (*Capra pyrenaica*) populations from Andalusia, in southern Spain. The predisposing factors to infection among previously selected factors (year of sampling, climatic season, geographic origin according to province, mountain range and metapopulation, sex, year of life, presence of scabies, and phase of the reproductive cycle) were established. We collected conjunctival and ear-canal swabs from 411 free-ranging ibexes. The frequency of infected ibexes was 11.2%. The peak frequency of infection occurred in 1998 and in summer. Granada was the province with greatest risk (odds ratio=2.6) of carriers (18.8% infected). The predisposing factors were sex (females), age (young animals), and metapopulation (Sierra Nevada). We identified a higher number of infected ibexes in the metapopulation "Sierra Nevada" (34/256) and significant differences among the three established metapopulations ($P<0.01$). *Mycoplasma agalactiae* infection represents a risk for population density and maintenance of these wild populations; infections can result in blindness, malnutrition, and polyarthritis leading to numerous deaths.

Key words: *Capra pyrenaica*, contagious agalactia, epidemiology, infectious diseases, *Mycoplasma agalactiae*, risk factors, Spanish ibex.

INTRODUCTION

The Spanish ibex (*Capra pyrenaica*) is the unique native Caprinae species on the Iberian Peninsula, and natural populations are found in a large portion of southern and eastern Spain. Andalusia is a region located in the south of Spain; it is delimited on the south by the Atlantic Ocean, on the north by the regions of Extremadura and Castilla La Mancha, on the east by the region of Murcia and by the Mediterranean Sea, and on the west by Portugal. Spanish ibexes are the most numerous in the Andalusian Mountain regions (Pérez et al., 2002), but since the mid-1980s, these populations have been affected by infectious diseases, such as sarcoptic mange, which have contributed to a significant population decline (León et al., 1999; Pérez et al., 1997, 2002). Few studies have investigated the demographic, ecologic, biologic, and genetic characteristics of the Spanish ibex in Andalusia; there also is limited information on the infectious diseases that may impact these populations (León et al., 1989, 1992a, 1992b; Pérez, 2001).

In Europe, Spain is ranked second for the number of goats, and Andalusia is the region primarily associated with goat production (Ministry of Agriculture and Fishing of Spain, 2005). Contagious agalactia syndrome (CAS) in small dairy ruminants is characterized by mastitis, arthritis, keratoconjunctivitis, and, occasionally, abortion (Nicholas, 2002) and pneumonia (Cokrevski et al., 2001). It has been reported worldwide and is endemic in most Mediterranean countries (Contreras et al., 2003). In Spain, CAS is the major cause of economic losses in dairy goat production, and it is widely distributed throughout the country, in-

cluding the Canary Islands (Real et al., 1994). *Mycoplasma agalactiae* is the most common agent associated with disease in small ruminants; in Spain, the species causes 90% of CAS outbreaks (Garrido et al., 1987).

Mycoplasma conjunctivae infection is most frequently associated with free-ranging Caprinae in European ibex, and there have been reports of affected alpine ibex (*Capra ibex ibex*), chamois (*Rupicapra rupicapra*), and wild sheep (*Ovis ammon*; Giacometti et al., 2002). Other mycoplasmal infections have been reported in captive wild ungulates, including a recently reported infection by "group *Mycoides*" mycoplasmas in Vaal rheboks (*Pelea capreolus*) (Nicolas et al., 2005) and *Mycoplasma capricolum* infection in an ibex (Schweighardt et al., 1989). We have previously reported various mycoplasmal infections in Spanish ibex populations (González-Candela et al., 2006); however, the present study is the first epidemiologic analysis of *M. agalactiae* infection in Caprinae. Because there are no data available about temporal, spatial, or host-related factors influencing *M. agalactiae* infection in natural populations of ungulates, this work contributes to the understanding of basic disease epidemiology, which will aid in the prevention and control of future CAS mycoplasmal infections in wild goats in Spain and other Mediterranean countries.

The purpose of this study was to establish the epidemiologic factors that influence *M. agalactiae* infection dynamics and that could act as predisposing factors to disease in the future.

MATERIALS AND METHODS

Temporal distribution of samples

Sampling was done from 1996 to 2003; however, no samples were collected during 1999–2001. Animals were sampled by veterinarians, biologists, and by the technical staff (veterinarians and wildlife personnel) working at the National Park of Sierra Nevada, in Granada and Almeria. Sample size varied by year (1996: $n=3$; 1997: $n=131$; 1998: $n=173$; 2002: $n=77$; 2003: $n=27$) and season (winter: $n=120$; spring: $n=177$; summer: $n=97$; autumn: $n=17$).

Spatial distribution of samples

The study was conducted on Spanish ibex populations from the massifs of the Andalusia region in southern Spain (36°N to 38°60′N, 1°75′W to 7°25′W). We analyzed 411 free-ranging ibex individuals originating from the mountain ranges of the provinces of Almería ($n=161$), Cádiz ($n=11$), Granada ($n=117$), Jaén ($n=31$), and Málaga ($n=91$).

For analyses, we established three metapopulations based on the geographic origin of the animals and the genetic studies conducted by Pérez et al. (2002). The most extended metapopulation (Sierra Nevada; $n=273$) included the mountain ranges of Sierras de la Contraviesa, Laujar, Lújar, Gádor, and Nevada. The metapopulation "Sierras de Jaén" ($n=30$) included the mountain ranges Sierras de Alta Coloma, Mágina, Los Canjorros, and Cazorla; Sierras de Málaga ($n=108$) included the mountain ranges of Málaga and Cádiz (Sierras de Tejeda-Almijara, Tejeda, Grazalema, Líjar, Los Alcornocales, Ronda, Bermeja, Alcaparaín, Aguas, Prieta, Ortegícar, Peñarrubia, De la Chimenea, Camarolos, Madroño, and Loja; Fig. 1).

Host-related factors

The sampled group included 275 males and 136 females. Female reproductive status was classified as estrus (October to January; $n=45$), pregnancy and parturition (February to May; $n=213$), and lactation (June to September; $n=153$).

Data and sample collection

Hunter-killed animals were sampled by forest agents; additional samples were taken from animals captured for scientific studies and from a few that were found dead. Conjunctival and ear-canal exudates were collected with sterile swabs using Amies medium with Charcoal (Venturi Transystem™, Copan Italia, Bovezzo, Italy) and refrigerated at 4 C during transport to the Infectious Diseases Laboratory of the Faculty of Veterinary Medicine at the University of Murcia. Samples were cultured for a maximum of 3 days after collection for the presence of mycoplasma using the recommendations described by Cottew (1983), Whitford (1994), and the OIE (2004).

Mycoplasmas were isolated in solid and

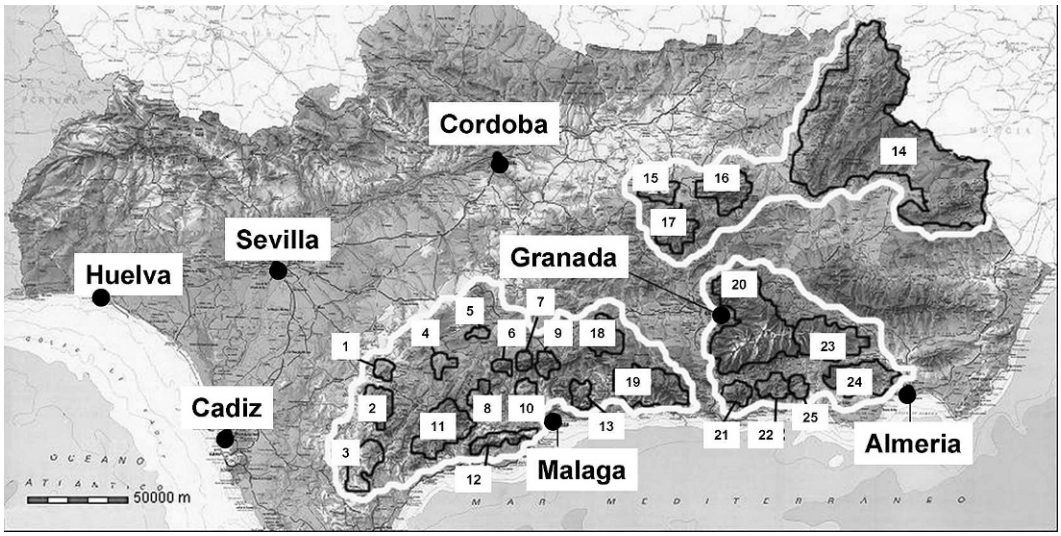

FIGURE 1. Geographic localization of metapopulations (bordered in white) sampled from 1996 to 2003. Mountain ranges (number of ibexes tested/number positive): 1, Líjar (1/0); 2, Grazalema (10/0); 3, Los Alcornocales (1/0); 4, Ortegícar (2/0); 5, Madroño (4/0); 6, Alcaparaín (2/0); 7, De la Chimenea (1/0); 8, Aguas (2/1); 9, Camarolos (2/1); 10, Prieta (2/0); 11, Ronda (38/0); 12, Bermeja (1/0); 13, Tejeda-Almijara (33/1); 14, Cazorla (27/1); 15, Mágina (1/0); 16, Los Canjorros (1/1); 17, Alta Coloma (2/0); 18, Loja (4/1); 19, Tejeda (1/0); 20, Nevada (Granada) (110/20); 21, Lújar (1/0); 22, Contraviesa (Granada) (1/1); 23, Nevada (Almeria) (146/14); 24, Gádor (8/3); 25, Contraviesa (Almeria) (5/2).

liquid forms of selective modified Hayflick's medium. Briefly, the medium consisted of: standard mycoplasma PPLO Growth medium™ (Difco Laboratories, Detroit, Michigan, USA) enriched with 16% horse serum, 0.5% yeast extract, 0.5% glucose, and 0.6% brain-heart infusion (Difco Laboratories), supplemented with arginine, cysteine, nicotinamide, deoxyribonucleic acid (0.05%), and 0.1 mg/ml of sodium ampicillin. Plates with solid medium and 2.0 ml of liquid medium were inoculated directly and incubated 3 to 7 days at 37 C in a humid chamber with 10% CO_2. At days 7 and 14, 0.2 ml of liquid and solid media were reinoculated on new media under the same conditions; total incubation time was 21 days. Plates were observed daily, and when mycoplasmal growth was visualized microscopically, isolates were cloned and identified.

The biochemical profile (sensitivity to digitonin, hydrolysis of urea, fermentation of glucose, hydrolysis of arginine, phosphatase activity, film and spots production, tetrazolium reduction, liquefaction of inspissated serum, and hydrolysis of casein) of the mycoplasma isolates, which were cloned four times, was determined using the methods of Aluotto et al. (1970). The growth inhibition test was done using hyperimmune antisera obtained from rabbits (against the reference strains of caprine mycoplasmas that are used for serologic identification) as recommended by Poveda and Nicholas (2000).

Strains identified as *M. agalactiae* were analyzed by polymerase chain reaction (PCR) to confirm their identity. The technique was based on a fragment of the 16S rRNA gene as described by Chávez-González et al. (1995). Some modifications of this technique were made; briefly, we used 2.5 µl of sample, 2.5 U of Taq DNA polymerase, 10 mM of Tris-HCl (pH 9.0), 50 mM of KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 µl of 2.5 µM of each primer, and 25 µl of total amount of mixture. The PCR protocol included 35 amplification cycles using a Tpersonal 48™ model (Whatman Biometra, Goettingen, Germany) and a hot start for 5 min at 94 C. Cycles consisted of an initial denaturation for 45 sec at 94 C, 1 min at 60 C, and 2 min at 72 C. The final extension step consisted of 10 min at 72 C. Extraction of genomic DNA from cultures and visualization of PCR products were done as described in Chávez-González et al. (1995).

Six *M. agalactiae* isolates and the type strain PG2 were sequenced at the DNA Sequencing Service of the University of Murcia. The sequences were analyzed with Chromas Lite© program (Technelysium Pty. Ltd., Australia) and compared with sequences deposited

in GenBank (NCBI) using the program BLASTN 2.2.11 (Altschul et al., 1997).

Statistic analysis

Risk factors potentially associated with the prevalence of *M. agalactiae* used in the analyses included year of sampling, climatic season, province, mountain ranges, metapopulation, sex, age, presence of scabies, and the reproductive cycle. Statistic analyses were performed with Microsoft EXCEL 2000© (1985–1999, Microsoft Corporation, USA) and EpiInfo 3.3.2 (Centers for Disease Control, Atlanta, Georgia, USA, 2005) using Pearson's chi-square test without correction and the Fisher's exact test. Two-tailed tests at a significance level of $P \leq 0.05$ were used. Odds ratios (OR) were calculated using Cornfield 95% confidence limits (EpiInfo 3.3.2; Centers for Disease Control, USA, 2005).

Finally, we included the statistically significant factors in a logistic regression model to identify predisposing risk factors. The initial formula was: $\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n$. When the data were recovered in the original scale by transforming p, the final formula of the regression was: $p = \frac{e^{\beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n}}{1 + e^{\beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n}}$, where ln is the Neperian logarithm, p is the probability, a is a constant, b is a regression coefficient, x is the independent variable, and e is the exponential.

RESULTS

Identification of *M. agalactiae* and presence of scabies

Mycoplasma agalactiae was isolated from 46 of the 411 (11.2%) Spanish ibexes. Sequences comparison made using the program BLASTN 2.2.11 (Altschul et al., 1997) confirmed the identity of the six strains sequenced as *M. agalactiae*.

Fifty Spanish ibex were infected by *Sarcoptes scabiei*; six of these were infected with *M. agalactiae*. There was no significant association between the two infections; scabies did not represent a risk factor in *M. agalactiae* infection ($OR=1.09$; $0.4<OR<2.6$). The six coinfected animals came from the Granadine portion of Sierra Nevada mountain range.

Temporal-spatial distribution of *M. agalactiae* infections

Significant differences in prevalence of *M. agalactiae* were detected between years ($\chi^2=31.29$; df=4; $P<0.01$); data from 1996, when only three ibex were sampled, and 1998, when there was an epidemic of CAS in ibex in Almeria and Granada (21.4% infected) were excluded from the analysis (Table 1). There was no statistic relationship detected between climate and *M. agalactiae* prevalence, although the highest frequency of infected ibex was observed in summer and spring (Table 1).

The highest prevalence of infected animals was observed in Granada (22/46; 48%; ($P<0.01$)). In Almeria, 19 infected ibex (19/46; 41%) were observed. Although not significantly different, the lowest prevalence was observed in Malaga (Table 2).

Most of the infected ibex (34/46, 74%) were from the Sierra Nevada mountain range (SN), which extends along two provinces, Granada (GR) and Almeria (AL). In this range, there were 20 infected ibexes in Granada and 14 in Almería. In bordering mountain ranges in the southwest and east, including the Sierra de la Contraviesa, which extends along Almeria and Granada and the Sierra de Gador in Almeria, two and three additional *M. agalactiae* infected ibex were detected, respectively (Fig. 1).

Distribution by metapopulation

As in the mountain-range analysis, the highest prevalence of infection (40/273; 14.6%) was observed in the Sierra Nevada metapopulation ($OR=3.78$; $1.48<OR>10.19$), and there were significant differences between observed prevalences in each metapopulation ($\chi^2=9.99$; df=2; $P<0.01$). The lowest prevalence (4/108) was observed in the Sierras de Malaga metapopulation ($OR=0.24$; $0.7<OR>0.72$; $P<0.01$). The infection rate for the Sierras de Jaen metapopulation was 6% (2/30).

TABLE 1. Analysis of the association between the risk factors "year of study" and "climatic season" and *Mycoplasma agalactiae* infection in free-ranging Spanish ibexes from Andalusia, Spain (1996–2003).

Risk factor	*n*	Number infected	χ^{2b}	*P*	OR	OR ($CI_{95\%}$) Minimum	OR ($CI_{95\%}$) Maximum
Year							
1997	131	5	10.52	<0.01	0.23	0.08	0.63
1998[a]	173	37	31.24	<0.01	6.92	3.09	15.94
2002	77	3	5.07	0.02	0.27	0.07	0.95
2003	27	1	–	NS[c]	0.29	0.01	2.08
Climatic season							
Winter	120	12	0.24	NS	0.84	0.39	1.76
Spring	177	17	0.79	NS	0.75	0.38	1.47
Summer	97	16	3.59	0.06	1.87	0.92	3.76
Autumn	17	1	–	NS[c]	0.48	0.02	3.61

[a] Contagious agalactia syndrome (CAS) outbreak.

[b] Pearson's χ^2 without correction.

[c] Fisher's exact test; NS = nonsignificant.

Host factors influencing infection

Prevalence rates of infection in males (8.4%) and females (16.9%) were significantly different ($\chi^2=6.69$; df=1; $P<0.01$). Females were more at risk of infection (OR=2.23; 1.15<OR>4.33), whereas the males showed a tendency to be protected against *M. agalactiae* infection (OR=0.45; 0.23<OR>0.87).

A significant association was detected between age (year of life) and *M. agalactiae* infection (Table 3). Most (32/46: 70%) of the infected animals were in the year-one to year-four age classes. The highest prevalence was detected in the year-three age class (11/41, 27%). In the older age classes, prevalence was generally low; the only exception was in the 13-yr-old animals.

Infection was related to reproductive cycle ($\chi^2=8.42$; df=2; $P<0.01$). The greater frequency of infection was observed during the lactation cycle (Table 4).

Predisposing factors: Multifactor analysis

The explanatory variables *metapopulation*, *sex*, and *age* were included in the logistic regression equation. The regression equation applied for the graphic projection of the model was:

$$\Pr M.\,ag. = \frac{e^{(a + b_{sex}x_{sex} + b_{year\ of\ life}x_{year\ of\ life} + b_{metapopulation}x_{metapopulation})}}{1 - e^{(a + b_{sex}x_{sex} + b_{year\ of\ life}x_{year\ of\ life} + b_{metapopulation}x_{metapopulation})}},$$

TABLE 2. Analysis of the association between the risk factor "province" and *Mycoplasma agalactiae* infection in free-ranging Spanish ibex from Andalusia, Spain (1996–2003).

Province	*n*	Number infected	χ^{2a}	*P*	OR	OR ($CI_{95\%}$) Minimum	OR ($CI_{95\%}$) Maximum
Almería	161	19	0.10	0.75	1.11	0.57	2.15
Cádiz	11	0	–	NS[b]	0.00	0.00	3.79
Granada	117	22	9.53	<0.01	2.61	1.34	5.08
Jaén	31	2	–	NS[b]	0.53	0.08	2.38
Málaga	91	3	7.33	<0.01	0.22	0.22	0.76

[a] Pearson's χ^2 without correction.

[b] Fisher's exact test; NS = nonsignificant.

TABLE 3. Analysis of the association between the risk factor "year of life" and *Mycoplasma agalactiae* infection in free-ranging Spanish ibexes from Andalusia, Spain (1996–2003).

Year of life	*n*		Number infected	χ^{2a}	*P*	OR	OR ($CI_{95\%}$)	
	Females	Males					Minimum	Maximum
1	30	32	10	1.79	NS	1.67	0.73	3.77
2	7	15	4	–	NS[b]	1.84	0.5	6.14
3	16	25	11	11.2	<0.01	3.51	1.51	8.07
4	12	30	7	–	NS[b]	1.69	0.64	4.32
5	5	21	1	–	NS[b]	0.3	0.01	2.17
6	20	39	3	2.59	NS	0.38	0.09	1.35
7	5	29	2	–	NS[b]	0.47	0.08	2.13
8	11	23	2	–	NS[b]	0.47	0.08	2.13
9	2	32	2	–	NS[b]	0.47	0.08	2.13
10	3	12	–	–	NS[b]	0.00	0.00	2.68
11	4	10	–	–	NS[b]	0.00	0.00	2.89
12	7	4	–	–	NS[b]	0.00	0.00	3.79
13	8	1	4	–	0.01[b]	6.86	1.48	30.95
14	1	2	–	–	NS[b]	0.00	0.00	18.19
15	4	0	–	–	NS[b]	0.00	0.00	12.47
18	1	0	–	–	NS[b]	0.00	0.00	140.0
Total	136	275	46	–	–	–	–	–

[a] Pearson's χ^2 without correction.

[b] Fisher's exact test; NS = nonsignificant.

where Pr *M. ag.* is the probability of *M. agalactiae* infection, *e* is the exponential, *a* is the equation constant, *b* is the coefficient of independent variables, and *x* is the value of independent variables. The results of the equation indicated a significant relation between the original metapopulation and the life year, as well as sex (Table 5).

In all metapopulations, young ibex had a significantly higher probability of *M. agalactiae* infection. Females in the Sierra Nevada metapopulation also had a higher probability of infection. Males in the Sierra Nevada metapopulation did have a higher prevalence of infection than males in the other metapopulations; prevalence also was higher than values observed in females of Sierras de Malaga metapopulation (Fig. 2).

DISCUSSION

In Spain, *M. agalactiae* is responsible for 90% of CAS outbreaks in domestic sheep and goats (Garrido et al., 1987; Rodriguez et al., 1996; Gil et al., 2003; De la Fe et al., 2005). A similar situation exists with domestic goats and sheep in Andalusia (Villalba, 2005). In wild ruminants in

TABLE 4. Analysis of the association between the risk factor "reproductive phases" and *Mycoplasma agalactiae* infection in free-ranging Spanish ibexes from Andalusia, Spain (1996–2003).

Reproductive phase	*n*	Number infected	χ^{2a}	*P*	OR	OR ($CI_{95\%}$)	
						Minimum	Maximum
Estrus	45	1	–	NS[b]	0.16	0.01	1.13
Pregnancy and parturition	213	20	1.45	NS	0.69	0.35	1.33
Lactation	153	25	6.5	<0.01	2.2	1.14	4.28

[a] Pearson's χ^2 without correction.

[b] Fisher's exact test; NS = nonsignificant.

TABLE 5. Results from the logistic regression model for the effects of variables on *Mycoplasma agalactiae* infection in free-ranging ibex from Andalusia, Spain (1996–2003).

Variables	Coefficient	SE	Wald χ^2	df	*P*	OR
Sex[a]	1.1	0.34	10.56	1	<0.01	3.00
Year of life	−0.13	0.05	6.23	1	0.01	0.88
Metapopulation Sierras de Jaén	–	–	12.13	2	<0.01	–
Metapopulation Sierra Nevada	0.99	0.77	1.68	1	0.19	2.70
Metapopulations Sierras de Málaga	−0.86	0.91	0.90	1	0.34	0.42
Constant	−2.58	0.80	10.44	1	<0.01	0.08

[a] Females/males are included in the constant.

Spain, antibodies to *M. agalactiae* have been reported from Spanish ibexes in the province of Malaga, as well as roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) in Cadiz (León et al., 1992a, 1994). Based on these previous reports, the high caprine population density in the studied area (Ministry of Agriculture and Fishing of Spain, 2005), and the potential for interspecific transmission among domestic sheep and goats, deer, and the ibex, the relatively high frequency of positive cultures in Spanish ibex reported in this study is not unexpected.

In the last decade, scabies affected some populations of wild goats, mainly in the southern Spain, specifically in the Natural Park of Sierra Nevada (Granada and Almeria) (Pérez et al., 1997). Almost all ibex populations in Andalusia are affected by scabies, as reported for populations from the Natural Park of Sierras de Cazorla, Segura y Las Villas (León et al., 1989), or Sierra Mágina in Jaen (Palomares and Ruiz-Martinez, 1993). Other wild ruminant species such as red deer, fallow deer (*Dama dama*), and mouflon (*Ovis musimon*) (León et al.,

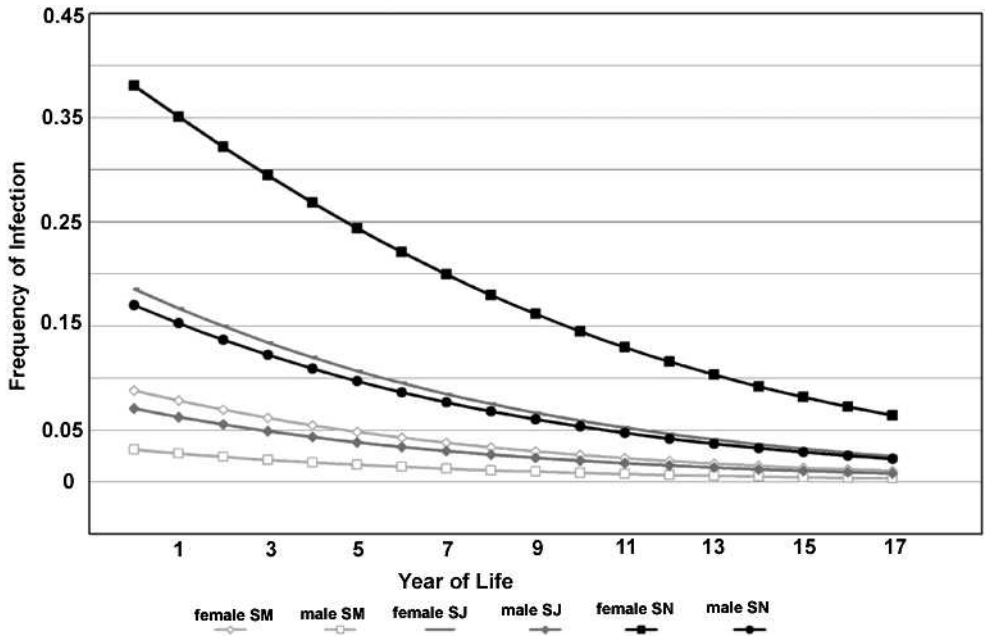

FIGURE 2. Trend of probability of *Mycoplasma agalactiae* infection in free-ranging Spanish ibexes based in a regression model including sex, age, and metapopulation as predisposing factors.

1992b) also are affected. Ibex may become immune suppressed as a result of scabies infections; however, in this study, no statistic association was observed between scabies and *M. agalactiae* infections. Because mange lesions are often difficult to observe in the field at extended distances, additional evaluations of this potential interaction are warranted.

Adverse climatic conditions, as well as other stress factors, also may influence diseases dynamics and the susceptibility of the animals to infections. Other factors, such as high population density, previous overpasturing, movement of infected domestic flocks (DaMassa et al., 1987; Kinde et al., 1994), and the presence of chronic or systemic diseases that compromise the immune system (DaMassa et al., 1987; Corrales et al., 2004) also may affect disease epidemiology. The annual report on the climate (Environmental Council of Regional Government of Andalusia, 2005) considered 1997 an anomalous and very warm year in Spain. In Andalusia, 1997 was characterized by abnormally high temperatures in winter and spring, followed by an increased relative humidity in summer; in 1998, there was a transition between a humid period and a severe period of drought in Andalusia. This climatic variability may explain why both of these years were identified as significant risk factors for *M. agalactiae* infection.

According to Chirosa et al. (2001), the population density of *C. pyrenaica* increased between 1996 and 1998. Since the year 2000, the National Park staff adopted a management policy to control population density in order to control the incidence of scabies within the population (Ministry of the Environment of Spain, 2005). The subsequent population reductions may have influenced the reduction in *M. agalactiae*–infected ibex observed in this study after 2000. The lower frequency of infected ibexes observed after 2000 also may have been related to the epidemiologic pattern of this disease; in domestic flocks, the infection usually develops a cyclical character due, in part, to the acquisition of temporary immunity (Bergonier et al., 1997). This same temporal pattern of infection in domestic goats was reported by Villalba (2005); between 1997 and 2001, the number of infected animals increased, and between 2002 and 2003, the prevalence of infection dramatically decreased. These consistent results observed in ibex and domestic goats imply some interspecific relationship, but additional and more detailed characterization of isolates is required to fully understand such potential interactions between wild and domestic species.

The period of lactation in ibex occurs in the summer, and both risk factors (season and reproductive phase) were associated with a higher frequency of infection. The association between lactation and infection by *M. agalactiae* is commonly observed in domestic flocks, which is consistent with intramammary transmission (Bergonier et al., 2003).

Information on the regional caprine production system in Andulusia may be needed to fully understand both the geographic pattern and the transmission dynamics of *M. agalactiae* in these domestic and wildlife populations. In eastern Andalusia, most goat production is associated with communal and rented pastures; this situation is different in western Andalusia, where goat herds are more restricted to individual pastures. The caprine production in Andalusia consists of a semi-extensive system of production (Public Company for Agricultural and Fishing Development of Andalusia, 2004), and it is possible that risks of transmission are higher (as observed in our study) in the eastern area (Almeria and Granada), where different herds share pasture. The geographic distribution of *M. agalactiae* also may be influenced by transhumance, which involves the transferring of livestock from one grazing ground to another (as from lowlands to highlands) with the changing of season. Transhumance occurs due to the regional

pasture and climatic conditions and can increase the probability of domestic and wild flocks sharing pastures in summer and winter. According to Bergonier et al. (1997), two major factors govern the evolution of the prevalence of *M. agalactiae* in domestic sheep and goats: the physiologic status of females and the movement of animals linked to transhumance, which promotes multiple contacts and stress. As the domestic animals and wildlife share pastures during transhumance, increased transmission to wildlife may occur. Such a relationship has been previously demonstrated with the transmission of *Mycoplasma conjunctivae* between domestic and wild small ruminants that share habitat in the Alps (Belloy et al., 2003). Our results demonstrated an association with mountain range; a high prevalence of *M. agalactiae* was observed in the Sierra Nevada. This association may be related to the combined effects of movement activities associated with transhumance and to increased ibex population density in the National Park of Sierra Nevada. The ibex population of the Natural Park of Sierra Nevada is very dense and larger than other populations—values ranged between 6.5 and 8.2 animals/km^2 until 2000 (Pérez et al., 2002).

Of the three metapopulations of *C. pyrenaica* included in this study, the Sierra Nevada metapopulation is the most genetically diverse (Manceau et al., 1999). This metapopulation maintains seven of the 10 haplotypes that characterize the Andalusian populations (Pérez et al., 2002); the population from Malaga and Cadiz, which were included in the metapopulation Sierras de Málaga, only maintains two of the 10 haplotypes. In domestic caprines, genetic factors linked to some breeds of dairy goats can influence susceptibility to intramammary infections (Barillet et al., 2001; Rupp and Boichard, 2003). It is therefore possible that the susceptibility of Spanish ibex to diseases such as contagious agalactia may be linked to genetic differences. Related to these potential genetic differences, our prediction model suggests that the female population of Sierra Nevada has a greater probability of infection. In the Sierra Nevada metapopulation, six exclusive haplotypes are present, but the most common haplotype also is present in the Sierras de Jaén metapopulation (Ministry of the Environment of Spain, 2005). Females from the Sierras de Jaén metapopulation had the second greatest probability of *M. agalactiae* infection, after the Sierra Nevada population.

There is little published information on the potential effects of gender as a risk factor for *M. agalactiae*. Domestic sheep and goats of both sexes can be infected at the same frequency (Madanat et al., 2001), but morbidity is most often associated with pregnant and lactating females rather than males (Ruffin, 2001); this probably relates to changes in immunologic competence caused by physiologic and hormonal changes associated with reproduction (Real et al., 1994). Lactating females often have morbidity rates between 10% and 90% (Bergonier et al., 1997), and in our study, females also had a higher frequency of infection. *Mycoplasma agalactiae* is transmitted orally (Hasso et al., 1994), and, based on this transmission route, differences in infection rates between sexes should not occur at young ages (Jones, 1987). However, there may be gender-related behaviors in ibex that are not present in the domestic sheep and goat herds that limit direct comparisons between these populations. In ibex populations, males are generally segregated except during the mating season, and, in general, the populations of Spanish ibex organize in groups of females with kids and young goats that have limited contact with groups of adult males (Alados, 1985). In spite of this statistic correlation and the low frequency of infection observed in males, it is important to recognize that males are infected and may play an important role in the epidemiology and the maintenance of *M. agalactiae*; most of

the isolates from males came from the ear canal (otic exudates), and infected animals did not exhibit any clinical signs related to these infections.

Other factors that may affect the epidemiology of *M. agalactiae* in Spanish ibex populations include population imbalances characterized by a sex- and age-ratio disparity favoring males, subadults, and old animals (Chirosa et al., 2001), anthropogenic factors, such as increased hunting pressure, and habitat-related anthropogenic factors, such as greater tourism in the Sierra Nevada regions (Ministry of the Environment of Spain, 2005). These factors have been linked to disease problems in wild sheep populations (Fedosenko and Weinberg, 1999; Sfougaris et al., 1999).

Further research on the molecular epidemiology of *M. agalactiae* in both domestic and wild Caprinae of the region will be necessary to fully understand the epidemiology of this disease and confirm the origin of the infection in Spanish ibexes. The infection in wild Caprinae populations could affect population dynamics in the Andalusian region, and Spanish ibex could act as a mycoplasma carrier, maintaining the pathogen in the environment and increasing the risk of infection for other wild ruminants as well as domestic goat flocks. A better understanding of *M. agalactiae* epidemiology therefore is needed to effectively manage these populations, especially in areas such as the National Park of Sierra Nevada.

ACKNOWLEDGMENTS

This investigation was part of the multidisciplinary project "Andalusian Plan of Survey and Control of Scabies in the Spanish Ibex Populations" funded by the Environment Council of Andalusia, which allowed studies on the population dynamics, infectious and parasite diseases, and genetic status of the Andalusian ibexes. We extend special thanks to A. Perales and F. Garrido (National Animal Health Laboratory, Santa Fe, Granada) for the microbiologic identification and all the personnel of the Zoology and Physical Anthropology Area (Faculty of Biology, University of Murcia) for their orientation over molecular identification. We also thank G. González Barberá (Center of Edaphology and Applied Biology of the Segura River, Consejo Superior de Investigaciones Cientificas, Murcia) for his help with the statistic analysis and all the field staff involved in data collection since 1996. Additional funding was provided by the Agriculture Council of Andalusia and the Animal Health Department of the University of Murcia.

LITERATURE CITED

Alados, C. L. 1985. Distribution and status of Spanish ibex (*Capra pyrenaica*). *In* The biology and management of mountain ungulates, S. Lovari (ed.). Croom-Helm, Beckenham, UK, pp. 204–211.

Altschul, S. F., T. L. Madden, A. A. Sháffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PST-BLAST. A new generation of database search programs. Nucleic Acid Research 25: 3389–3402.

Aluotto, B. B., R. E. Wittler, C. O. Williams, and J. E. Faber. 1970. Standardized bacteriologic techniques for the characterization of mycoplasma species. International Journal of Systematic Bacteriology 20: 35–58.

Barillet, F., P. Rupp, S. Mignon-Grasteau, J. M. Astruc, and M. Jacquin. 2001. Genetic analysis for mastitis resistance and milk somatic cell score in French Lacaune dairy sheep. Genetic Selection Evolution 33: 397–415.

Belloy, L., M. Janovsky, E. Vilei, P. Pilo, M. Giacometti, and J. Frey. 2003. Molecular epidemiology of *Mycoplasma conjunctivae* in Caprinae: Transmission across species in natural outbreaks. Applied and Environmental Microbiology 69: 1913–1919.

Bergonier, D., X. Berthelot, and F. Poumarat. 1997. Contagious agalactia of small ruminants: Current knowledge concerning epidemiology, diagnosis and control. Revue Scientifique et Technique de l'Office International des Épizooties 16: 848–873.

———, R. Crémoux, R. Rupp, G. Lagriffoul, and X. Berthelot. 2003. Mastitis of dairy ruminants. Veterinary Research 34: 689–716.

Centers for Disease Control, USA. 2005. *Epi Info* 3.3.2, http://www.cdc.gov/epiinfo/. Accessed at 16 March 2005.

Chávez-González, Y., C. R. Bascuñana, G. Bölske, J. Mattsson, C. F. Molina, and K. E. Johansson. 1995. In vitro amplification of the 16S rRNA genes from *Mycoplasma bovis* and *Mycoplasma agalactiae* by PCR. Veterinary Microbiology 47: 183–190.

Chirosa, M., J. R. Delibes-Senna, P. E. B. Fandos, J. E. Granados, M. C. Pérez, J. M. Pérez, I. Ruíz-Martínez, E. Serrano, R. C. Soriguer, and

S. Weykan. 2001. Estimas poblacionales de *Capra pyrenaica hispanica*. *In* Distribución, genética y estatus sanitario de las poblaciones de cabras montesas. Andalusia Council and University of Jaén, Jaén, Spain, pp. 3–68.

Cokrevski, S., D. Crcev, G. R. Loria, and R. A. J. Nicholas. 2001. Outbreaks of contagious agalactia in small ruminants in the Republic of Macedonia. Veterinary Record 148: 667.

Contreras, A., C. Luengo, A. Sanchez, and J. C. Corrales. 2003. The role of intrammamary pathogens in dairy goats. Livestock Production Science 79: 273–283.

Corrales, J. C., A. Contreras, and A. Sánchez. 2004. Mamitis caprinas y agalaxia contagiosa. *In* Ganado Caprino—Producción, Alimentación y Sanidad, A. Daza, C. Fernández and A. Sánchez (eds.). Editorial Agrícola Española S.A., Madrid, Spain, pp. 271–292.

Cottew, G. S. 1983. Recovery and identification of caprine and ovine mycoplasmas. *In* Methods in mycoplasmology, Vol. II, S. Razin and J. G. Tully (eds.). Academic Press Inc., New York, New York, pp. 91–104.

DaMassa, A. J., D. L. Brooks, C. A. Holmberg, and A. I. Moe. 1987. Caprine mycoplasmosis: An outbreak of mastitis and arthritis requiring the destruction of 700 goats. Veterinary Record 120: 409–413.

De la Fe, C., P. Assunao, T. Antunes, R. S. Rosales, and J. B. Poveda. 2005. Microbiological survey for *Mycoplasma* spp. in a contagious agalactia endemic area. The Veterinary Journal 170: 257–259.

Environmental Council of Regional Government of Andalusia. 2005. *Medio ambiente en Andalucía/Informe 1997 y Informe 1998*, http://www.juntadeandalucia.es/medioambiente. Accessed 6 October 2005.

Fedosenko, A. K., and P. J. Weinberg. 1999. The status of some wild sheep populations in the CIS (former URSS) and the impact of trophy hunting. Caprinae—Newsletter of the International Union for Conservation Nature/Species Survival Commission Caprinae Specialist Group: 1–4.

Garrido, F., L. León, J. L. Ladero, L. Cuellar, and M. A. Díaz. 1987. Contagious agalactia in Spain. *In* Contagious agalactia and other mycoplasmal diseases of small ruminants, M. Lambert and G. Jones (eds.). Office for Official Publications of the European Communities, Commission of the European Community, Luxembourg, pp. 1–6.

Giacometti, M., M. Janovsky, J. Jenny, J. Nicolet, L. Belloy, E. Goldschmidt-Clermont, and J. Frey. 2002. *Mycoplasma conjunctivae* infection is not maintained in alpine chamois in eastern Switzerland. Journal of Wildlife Diseases 38: 297–394.

Gil, M. C., F. G. Peña, J. Hermoso de Mendoza, and L. Gómez. 2003. Genital lesions in an outbreak of Caprine contagious agalactia caused by *Mycoplasma agalactiae* and *Mycoplasma putrefaciens*. Journal of Veterinary Medicine B50: 484–487.

González-Candela, M., G. Verbisck-Bucker, P. Martín-Atance, M. J. Cubero-Pablo, and L. León-Vizcaíno. 2007. Mycoplasmas carried by Spanish ibex (*Capra pyrenaica*) in southern Spain: Frequency and risk factors associated. Veterinary Record 161: 167–168.

Hasso, S. A., A. M. Al-Darraji, and J. M. Al-Aubaidi. 1994. Pathology of experimentally induced contagious agalactia in goats. Small Ruminant Research 13: 79–84.

Jones, G. E. 1987. Contagious agalactia and other mycoplasmal diseases of small ruminants. Commission of European Communities, Luxemburg, pp. 1–118.

Kinde, H., A. J. DaMassa, P. S. Wakenell, and R. Petty. 1994. Mycoplasma infection in a commercial goat dairy caused by *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* (caprine biotype). Journal of Veterinary Diagnostic Investigation 6: 423–427.

León, L., F. Alonso, A. Contreras, M. J. Cubero, and R. Astorga. 1989. Brote de sarna sarcóptica (*Sarcoptes scabiei*) en cabra montés (*Capra pyrenaica*) en Cazorla. *In* Abstracts: IV Congreso Nacional y I Congreso Ibérico de Parasicología, I. Navarrete (ed.). Iberian Society of Parasitology, Madrid, Spain, pp. 316.

———, D. De Menegui, P. G. Meneguz, S. Rosati, and L. Rossi. 1992a. Encuesta seroepidemiológica de infecciones en la población de cabra montés del Parque Natural de la Sierra de la Nieves (Ronda, Málaga). Junta de Andalucia, Actas del V Congreso Internacional del Género Capra, Ronda, Málaga, Spain, 20–22 October, pp. 95–100.

———, R. Astorga, J. Escós, F. Alonso, C. Alados, A. Contreras, and M. J. Cubero. 1992b. Epidemiología de la sarna sarcóptica en el Parque Natural de las Sierras de Cazorla, Segura y Las Villas. *In* Proceedings of the International Congress of the Genus *Capra* in Europe. Director Council of Natural Park of Sierra de las Nieves, Environmental Council of Regional Government of Andalusia, Sevilla, Spain, pp. 95–99.

———, ———, and M. J. Cubero. 1994. Aproximación al estado sanitario de los corzos Andaluces. *In* El corzo Andaluz, F. Braza, S. San José, S. Aragón and J. R. Delibes (eds.). Environmental Council of Regional Government of Andalusia, Sevilla, Spain, pp. 85–97.

———, M. R. Ruiz, M. J. Cubero, J. Ortiz, J. Espinosa, L. Pérez, M. Simón, and F. Alonso. 1999. Sarcoptic mange in Spanish ibex from Spain. Journal of Wildlife Diseases 35: 647–659.

MADANAT, A., D. ZENDULKOVÁ, AND Z. POSPÍSIL. 2001. Contagious agalactia of sheep and goats. A review. Acta Veterinaria Brno 70: 403–412.

MANCEAU, V., J. P. CRAMPE, P. BOURSOT, AND P. TABERLET. 1999. Identification of evolutionary significant units in the Spanish wild goat, *Capra pyrenaica* (Mammalia, Artiodactyla). Animal Conservation 2: 33–39.

MINISTRY OF AGRICULTURE AND FISHING OF SPAIN. 2005. *Ganado ovino y caprino. Censos*, http://www.mapya.es/. Accessed 6 July 2005.

MINISTRY OF THE ENVIRONMENT OF SPAIN. 2005. *Plan Especifico de Gestión de las Poblaciones de Cabra Montés* (Capra pyrenaica) *en el Parque Nacional de Sierra Nevada*, http://www.mma.es/parques/lared/informes/sn_fau_cabra.pdf Accessed 15 June 2005.

NICHOLAS, R. A. J. 2002. Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. Small Ruminant Research 45: 145–149.

NICOLAS, M. M., I. H. STALIS, T. L. CLIPPINGER, M. BUSCH, R. NORDHAUSEN, G. MAALOUF, AND M. D. SCHRENZEL. 2005. Systemic disease in Vaal rhebok (*Pelea capreolus*) caused by mycoplasmas in the mycoides cluster. Journal of Clinical Microbiology 43: 1330–1340.

Office International des Epizooties. 2004. Contagious agalactia. *In* Manual de las pruebas de diagnostico y de las vacunas para los animales terrestres (mamíferos, aves y abejas), Vol. II. World Organization for Animal Health, Paris, France, pp. 653–660.

PALOMARES, F., AND I. RUIZ-MARTINEZ. 1993. Status und aussichten für den Schutz der Population des Spanischen Steinbocks (*Capra pyrenaica*) im Sierra Magina NaturPark in Spanien. Journal of Jagdwissen 39: 87–94.

PÉREZ, J. 2001. Introducción. *In* Distribución, genética y estatus sanitario de las poblaciones Andaluzas de cabra montés. Environment Council, Regional Government of Andalusia and University of Jaén, Jaén, Spain, pp. 276.

———, I. RUIZ-MARTINEZ, J. GRANADOS, R. SORIGUER, AND P. FANDOS. 1997. The dynamics of sarcoptic mange in the ibex population of Sierra Nevada in Spain—Influence of climatic factors. Journal of Wildlife Research 2: 86–98.

———, J. E. GRANADOS, R. C. SORIGUER, P. FANDOS, F. J. MARQUEZ, AND J. P. CRAMPE. 2002. Distribution, status and conservation problems of the Spanish ibex, *Capra pyrenaica* (Mammalia: Artiodactyla). Mammal Review 32: 26–39.

POVEDA, J. B., AND R. NICHOLAS. 2000. Serological identification of mycoplasmas by growth and metabolic inhibition tests. *In* Methods in molecular biology, Vol. IV, Mycoplasma protocols, J. Miles and N. Nicholas (eds.). Humana Press Inc., New York, New York, pp. 105–111.

PUBLIC COMPANY FOR AGRICULTURAL AND FISHING DEVELOPMENT OF ANDALUSIA. 2004. *Producción animal*, http://www.dap.es/Empresa_Presentacion.aspx. Accessed 21 March 2004.

REAL, F., S. DÉNIZ, B. ACOSTA, O. FERRER, AND J. B. POVEDA. 1994. Caprine contagious agalactia caused by *Mycoplasma agalactiae* in the Canary Islands. Veterinary Record 135: 15–16.

RODRIGUEZ, J. L., J. B. POVEDA, F. RODRIGUEZ, A. ESPINOSA DE LOS MONTEROS, A. S. RAMIREZ, AND A. FERNANDEZ. 1996. Ovine keratoconjunctivitis caused by *Mycoplasma agalactiae*. Small Ruminant Research 22: 93–96.

RUFFIN, D. C. 2001. Mycoplasma infections in small ruminants. Veterinary Clinics of North America: Food Animal Practice 17: 315–332.

RUPP, R., AND D. BOICHARD. 2003. Genetic resistance to mastitis in dairy cattle. Veterinary Research 34: 671–688.

SCHWEIGHARDT, H., P. PECHAN, E. LAUERMANN, AND G. KRASSNIG. 1989. *Mycoplasma capricolum* infection in an alpine ibex (*Capra ibex ibex*)—Eine Fallbeschreibung. Kleintierpraxis 34: 297–299.

SFOUGARIS, A. I., A. GIANNAKOPOULOS, H. GOUMAS, AND E. TSACHALIDIS. 1999. Status and management needs of a Balkan chamois population in the Rodopi Mountains. Caprinae—Newsletter of the International Union for Conservation Nature/Species Survival Committee Caprinae Specialist Group: 4–5, May 1999.

VILLALBA, E. J. 2005. Estudio de infecciones por microorganismos de la Clase *Mollicutes* en pequeños rumiantes de la Comunidad Autónoma Andaluza. Doctoral Thesis, University Las Palmas de Gran Canaria, Gran Canaria, Spain, 293 pp.

WHITFORD, H. W. 1994. Isolation of mycoplasmas from clinical specimens. *In* Mycoplasmosis in animals: Laboratory diagnosis, H. W. Whitford, R. F. Rosenbusch and L. H. Lauerman (eds.). Iowa State University Press, Ames, Iowa, pp. 13–14.

Received for publication 13 November 2006.