

Original Article

Seroprevalence Survey of Zoonoses in Extremadura, Southwestern Spain, 2002–2003

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SUMMARY: Our aims were to determine the seroprevalence rates for the most common types of zoonosis among the population of Extremadura (southwestern Spain) and to identify the associated risk factors. We conducted a seroepidemiological survey to collect information on family background and the habits of people residing in Extremadura between 2002 and 2003. Antibodies to *Brucella* were determined by Rose Bengal staining and a standard tube agglutination test; a titer of 1/80 was considered to be positive. Antibody titers for spotted fever, leishmaniasis, echinococcosis, and toxoplasmosis were determined by enzyme-immunoassays. Independent risk factors identified were age (younger age for brucellosis), male gender (brucellosis, spotted fever, and toxoplasmosis), occupation and contact with animals (brucellosis and spotted fever for those in contact with goats, hydatidosis for those in contact with sheep, leishmaniasis for those in contact with dogs, and toxoplasmosis for those in contact with cats and pigs), and consuming contaminated food (brucellosis by eating fresh cheese, hydatidosis by eating homemade sausages, and toxoplasmosis by eating pork). Except for leishmaniasis, the other zoonoses were more prevalent in rural areas, and, with the exception of brucellosis, they were all more prevalent in Badajoz. The distribution of zoonoses in Extremadura was strongly influenced by keeping livestock and eating habits. Thus, brucellosis was more prevalent in Caceres (associated with cheese consumption), while toxoplasmosis (pork consumption) and spotted fever (from hunting) were more common in Badajoz.

INTRODUCTION

In recent years, zoonoses have become an important issue in public health due to their worldwide distribution, their incidence in humans, and the economic, health, and social problems associated with zoonoses (1–3). As a member of the European Union, Spain implements a common system of protective measures against certain types of zoonosis. These measures focus primarily on 2 aspects, a system for monitoring and reporting cases and programs to combat, control, and eradicate these types of zoonosis. Such measures have resulted in continued reduction in the number of cases reported nationwide (4).

However, since 2000, this positive development suffered a setback, with the appearance of active outbreaks of brucellosis in various regions of Spain (4). Thus, although between 2001 and 2009, the incidence of brucellosis in Extremadura declined from 7.17 to 0.93 cases per 100,000 inhabitants per year (4), Extremadura is an autonomous community with the most reported

cases in Spain, particularly in the province of Caceres, in which brucellosis occurs predominantly in small towns (5). The annual incidence of echinococcosis and spotted fever in this region is also generally higher than the national average (4).

Identifying of the sources of the foci for certain zoonoses that do not disappear (6) and assessing the epidemiological situation would aid in defining the actual impact of these diseases. To date, there have been no studies on the seroprevalence rates of zoonoses in representative samples of the general population; thus, the actual prevalence of these diseases and their association with recognized risk factors remain unknown.

Therefore, our aims in this study were to determine the seroprevalence rates for the most common types of zoonosis among humans living in Extremadura and to identify the risk factors associated with zoonosis.

METHODS

Target population: Our target population for this survey included any resident of Extremadura older than 24 months of age who had undergone a blood test in a Public Health Primary Care Clinic between 2002 and 2003.

Sampling type and size: A complex sampling design was used, with 2-stage equiprobabilistic clustering. The sample size was proportionally allocated by health area and district and stratified by age groups and gender. Extremadura is divided into 8 health areas, 4 in Badajoz (Badajoz, Merida, Don Benito-Villanueva, and Llerena-

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Zafra) and 4 in Cáceres (Cáceres, Coria, Navalmoral, and Plasencia), with 36 health districts in all. To determine the required sample size, a formula for simple random sampling was used, after applying a correction factor for design effect that was estimated to be 1.4. Participating health centers were randomly chosen through computer assignments of pseudorandom numbers, using the 1991 census as the reference population.

Zoonosis types and variables included in this study: Zoonoses of greater interest to the autonomous community of Extremadura were chosen. These included brucellosis, echinococcosis, Mediterranean spotted fever, leishmaniasis, and toxoplasmosis. A self-administered questionnaire was used to collect demographic data, personal and family histories, current and/or prior occupation and occupation of the head of household, any vaccines and pharmacological treatments received, contact with animals (sheep, goats, cows, dogs, and cats), outdoor excursions (hunting, fishing, etc.), tick bites, and consumption of unpasteurized milk or dairy products (cheese and/or homemade butter), homemade sausages, and big game meat (wild boar, deer). We excluded those people with any type of immunosuppressive disease and/or who had received immunosuppressive therapy during the 3 months prior to this study.

Serological techniques: The presence of IgG antibodies for *Brucella* was determined using a slide agglutination test with Rose Bengal staining (Difco, Madrid, Spain). To confirm the results and determine antibody titers, the tube agglutination standard test was performed with the same reagent; a titer of 1/80 was considered to be the threshold for a positive result. IgG antibody titers for the other zoonoses (spotted fever, leishmaniasis, toxoplasmosis, and echinococcosis) were determined using commercial enzyme immunoassays (Viracell, SL, Granada, Spain).

Data analysis: We evaluated our data using descriptive analyses. Point prevalence was estimated from the proportion (as determined from 95% confidence intervals) of the population that had antibodies directed against the diseases under study in each group (by age and gender), and stratified by the different variables ex-

amined.

Ethics statement: All experiments were in compliance with relevant laws and guidelines and in accordance with the ethical standards established in the Declaration of Helsinki.

RESULTS

A total 2,660 subjects were selected for this study. Their mean age was 32.2 years (SD: 23.8 years; range: 2–93 years). Our sample population included slightly more women (1,464; 55%) than men (1,196; 45%). The distributions for zoonosis prevalence by age and gender are shown in Table 1. Toxoplasmosis was the most prevalent zoonosis in Extremadura, particularly in the province of Badajoz, distantly followed by spotted fever and echinococcosis. In general, adults were more often affected than were children.

Brucellosis: This zoonosis primarily affected men (5-fold increased risk) between the ages of 20 and 50 years (Table 1). It was significantly related to occupation and contact with cattle (Table 2). Independent risk factors included male gender and having contact with goats and pigs (Table 3). Infection prevalence was higher in the health areas of Plasencia and Don Benito (Table 4), and predominantly in rural areas (Table 5).

Echinococcosis: No differences in prevalence were found for sex or age (Table 1). Actually, the only independent risk factors associated with this infection were consuming homemade sausages and contact with dogs (Table 3). Echinococcosis prevalence was higher in the health areas of Llerena and Navalmoral (Table 4), and mostly in rural areas (Table 5).

Spotted fever: This infection was slightly more prevalent among males aged 60 years and older (Table 1), and was significantly associated with occupation, tick bites, and contact with cows, goats, and pigs (Table 2). Independent risk factors included sex, age, tick bites, and contact with goats (Table 3). It was more prevalent in Badajoz and Llerena (Table 4), particularly in rural areas (Table 5).

Leishmaniasis: The prevalence of this infection was similar for both sexes, with a peak among those aged 60

Table 1. Seroprevalence of zoonosis analyzed by age and gender subgroups

Age (yr)	M (n)	F (n)	Brucellosis		Echinococcosis		Spotted fever		Leishmaniasis		Toxoplasmosis	
			M	F	M	F	M	F	M	F	M	F
2–4	90	96	0	0	0	3 (3.6%)	7 (7.3%)	6 (6.7%)	0	2 (2.2%)	5 (5.2%)	7 (7.8%)
5–9	152	178	2 (1.1%)	0	6 (3.4%)	7 (4.6%)	9 (5.1%)	6 (3.9%)	2 (1.1%)	1 (0.7%)	17 (9.6%)	11 (7.2%)
10–14	173	166	1 (0.6%)	0	6 (3.6%)	4 (2.3%)	8 (4.8%)	6 (3.5%)	3 (1.8%)	1 (0.6%)	13 (7.8%)	9 (5.2%)
15–19	168	104	1 (1%)	0	3 (2.9%)	4 (2.4%)	4 (3.8%)	3 (1.8%)	4 (3.8%)	2 (1.2%)	22 (21.2%)	21 (12.5%)
20–29	220	132	4 (3%)	1 (0.5%)	3 (2.3%)	4 (1.8%)	13 (9.8%)	10 (4.5%)	1 (0.8%)	1 (0.5%)	36 (27.3%)	43 (19.5%)
30–39	125	101	5 (5%)	0	5 (5%)	5 (4%)	7 (6.9%)	3 (2.4%)	1 (1%)	1 (0.8%)	38 (37.6%)	41 (32.8%)
40–49	121	110	4 (3.6%)	1 (0.8%)	4 (3.6%)	1 (0.8%)	8 (7.3%)	9 (7.4%)	1 (0.9%)	2 (1.7%)	58 (52.7%)	64 (52.9%)
50–59	131	78	0	0	4 (5.1%)	2 (1.55)	4 (5.1%)	11 (8.4%)	1 (1.3%)	1 (0.8%)	55 (70.5%)	80 (61.1%)
60–69	141	113	1 (0.9%)	0	3 (2.7%)	0	17 (15%)	8 (5.7%)	7 (6.2%)	7 (5%)	89 (78.8%)	109 (77.3%)
> 69	143	118	2 (1.7%)	1 (0.7%)	2 (1.7%)	1 (0.7%)	17 (14.4%)	10 (7%)	3 (2.5%)	4 (2.8%)	98 (83.1%)	119 (83.2%)
Total	1,464	1,196	20 (1.7%)	3 (0.2%)	36 (3%)	31 (2.1%)	94 (7.9%)	72 (4.9%)	23 (1.9%)	22 (1.5%)	431 (36.0%)	504 (34.4%)
Total	2,660		0.9%		2.5%		6.4%		1.7%		35.2%	

M, male/men; F, female/women.

Table 2. Frequency of exposure to various risk factors for the types of zoonoses analyzed

Risk factor	Brucellosis		Echinococcosis		Spotted fever		Leishmaniasis		Toxoplasmosis	
	N (2,637)	P (23)	N (2,593)	P (67)	N (2,494)	P (166)	N (2,615)	P (45)	N (1,725)	P (935)
High-risk profession ¹⁾	9.3%	52.4%*	9.7%	9.8%	9.3%	16.7%*	9.8%	7.9%	5.7%	15.9%*
High-risk profession for head of family	19.3%	45.5%*	19.5%	18.2%	19.5%	20.2%	19.5%	20.7%	16.8%	26.4%*
Non-sterilized milk	12.4%	21.7%	12.5%	13.4%	12.5%	12.7%	12.5%	15.6%	10.3%	16.6%*
Raw-milk cheese	46.8%	60.9%	46.8%	49.3%	47.2%	42.8%	46.8%	53.3%	43.0%	54.0%*
Homemade butter	2.7%	0%	2.5%	7.5%*	2.7%	2.4%	2.6%	8.9%*	3.0%	2.0%
Raw vegetables	40.9%	26.1%	40.6%	47.8%	40.9%	38.0%	40.8%	37.9%	38.6%	44.7%*
Homemade sausages	63.6%	87%	63.6%	74.3%	63.9%	63.3%	63.8%	66.7%	61.4%	68.2%*
Game	15.4%	34.8%*	15.5%	17.9%	15.5%	16.3%	15.6%	11.1%	14.8%	17.0%
Outdoor excursions	49.1%	65.2%	49.2%	50.7%	49.1%	50.6%	49.2%	48.9%	50.9%	46.1%*
Tick bites	6.6%	13%	6.7%	3.0%	6.3%	11.4%*	6.6%	8.9%	6.2%	7.5%
Contact with cows	9.5%	60.9%*	9.9%	9.0%	9.5%	16.9%*	9.9%	11.1%	9.3%	11.1%
Contact with sheep	12.7%	60.9%*	13.2%	13.4%	12.9%	17.5%	13.1%	15.6%	12.6%	14.1%
Contact with goats	7.5%	60.9%*	7.9%	7.5%	7.5%	15.1%*	7.8%	15.6%	7.2%	9.3%
Contact with pigs	17.1%	78.3%*	17.6%	17.9%	17.2%	23.5%*	17.7%	13.3%	16.2%	20.2%*
Contact with dogs	49.8%	65.2%	49.7%	59.7%	49.8%	52.4%	49.9%	53.3%	53.0%	44.4%*
Contact with cats	30.6%	65.2%*	30.9%	31.3%	30.6%	34.3%	30.8%	33.3%	30.6%	31.4%

¹⁾: High-risk professions: agricultural industry, animal husbandry, veterinarians, butchers, slaughterers.
N, negative; P, positive.

Table 3. Independent factors for seroprevalence of zoonosis in Extremadura

Risk factor	Brucellosis	Echinococcosis	Spotted fever	Leishmaniasis	Toxoplasmosis
	OR (CI 95%)	OR (CI 95%)	OR (CI 95%)	OR (CI 95%)	OR (CI 95%)
Sex (male vs. female)	5.71 (1.66–19.7)		1.58 (1.15–2.18)		1.21 (0.99–1.48)
Age (1 year increase)			1.013 (1.006–1.019)	1.021 (1.008–1.033)	1.066 (1.061–1.071)
High-risk profession					
Unsterilized milk					
Raw-milk cheese					
Homemade butter				3.98 (1.37–11.5)	
Raw vegetables					
Homemade sausages		1.61 (0.92–2.82)			
Game					
Outdoor excursions					
Tick bite			1.51 (0.89–2.55)		
Contact with cows					
Contact with sheep					
Contact with goats	7.08 (2.43–20.6)		1.86 (1.17–2.97)		
Contact with pigs	9.44 (2.69–33.1)		1.37 (1.04–1.80)		
Contact with dogs	0.31 (0.11–0.91)	1.42 (0.86–2.33)		1.36 (0.74–2.49)	
Contact with cats					1.22 (0.97–1.54)

OR, odds ratio; CI, confidence interval.

to 69 years (Table 1). The independent risk factors associated with this infection were age, consuming homemade butter, and contact with dogs (Table 3). The areas of highest prevalence were Mérida and Plasencia (Table 4), with a slight predominance in urban areas (Table 5).

Toxoplasmosis: This infection had a similar prevalence in both sexes, and the incidence of this zoonosis increased monotonically with age (Table 1). A statistically significant association was found between infection and an individual's occupation, consuming certain foods, such as raw milk, cheese, raw vegetables, and homemade sausage, and contact with pigs and dogs (Table 2). Identified independent risk factors were sex, age, and contact with pigs and cats (Table 3). The areas with

greatest toxoplasmosis prevalence were Badajoz and Llerena (Table 4), mostly in rural areas (Table 5). This zoonosis exhibited the greatest differences between the eight sample health areas, with a north-south and, to a lesser extent, a west-east gradient.

DISCUSSION

We found that the zoonoses investigated in our study were all associated with known risk factors, such as occupation, contact with animals, and consuming certain foods. Moreover, their incidence displayed an uneven geographical distribution, particularly toxoplasmosis that was most prevalent in Badajoz, and brucellosis, which was predominant in Cáceres. The seroprevalence

Table 4. Distribution of the seroprevalence for zoonosis by healthcare area

Healthcare area	Brucellosis		Echinococcosis	Spotted fever	Leishmaniasis	Toxoplasmosis ¹⁾
	<i>n</i>	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Badajoz	514	3 (0.6)	13 (2.5)	43 (8.4)	8 (1.6)	227 (44.2)
Cáceres	351	2 (0.6)	5 (1.4)	19 (5.4)	5 (1.4)	105 (29.9)
Coria	226	1 (0.4)	8 (3.5)	10 (4.4)	2 (0.9)	71 (31.4)
Don Benito	371	5 (1.3)	5 (1.3)	15 (4)	6 (1.6)	136 (36.7)
Llerena	253	3 (1.2)	11 (4.3)	21 (8.3)	3 (1.2)	97 (38.3)
Mérida	346	1 (0.3)	8 (2.3)	19 (5.5)	9 (2.6)	113 (32.7)
Navalmoral de la Mata	232	1 (0.4)	9 (3.9)	14 (6)	3 (1.3)	69 (29.7)
Plasencia	367	7 (1.9)	8 (2.2)	25 (6.8)	9 (2.5)	117 (31.9)

¹⁾: chi squared ($P < 0.01$).

Table 5. Seroprevalence for zoonosis in Extremadura by rural or urban area

	Rural (<i>n</i> = 1,785)	Urban (<i>n</i> = 857)	<i>P</i>
Brucellosis	19 (1.1%)	4 (0.5%)	0.12
Echinococcosis	54 (3%)	13 (1.5%)	0.02
Spotted fever	118 (6.6%)	46 (5.4%)	0.22
Leishmaniasis	27 (1.5%)	18 (2.1%)	0.27
Toxoplasmosis	644 (36.1%)	286 (33.4%)	0.17

rate of brucellosis was actually lower than expected based on reported cases, which emphasized that the seroprevalence rate of toxoplasmosis was the highest in this region (35%). Except for leishmaniasis, the other zoonoses were more prevalent in rural areas.

Brucellosis: Spain is one of the European Union countries that has the highest incidence of brucellosis, after Greece and Portugal (7), and Extremadura is an autonomous community with the highest incidence (8). This disease is primarily associated with *Brucella melitensis* and goat herding, although it is also associated with consuming fresh goat milk cheese (9). Based on our data, brucellosis was a zoonosis that was strongly associated with rural areas and occupation, as it was more frequently found among males who were in contact with goats or pigs. Gender is an important factor because those people with occupations that involve close contact with livestock are mostly men, those who are generally aged 30 to 40 years (10–12), and often involves various members of the same family (13). Our data showed that the prevalence of brucellosis was higher in those households for which the head of household had a high risk occupation.

The higher prevalence found in certain rural areas was due to disease outbreaks that occurred in recent years, which were mostly related to consuming raw-milk cheese (5,9,14). Despite measures that were established by the national brucellosis eradication program, the unauthorized sale of raw-milk cheese has made Extremadura a region with the highest incidence of this infection, with an overall prevalence of 0.9%. It is essential to debunk the mistaken belief that “homemade” is a guarantee for quality and raising awareness that these products can cause serious health problems. The prevalence of brucellosis that we found was similar to that found in other endemic areas (15), but lower than ex-

pected after taking into account the incidence data reported for this region (4). The epidemiological pattern of indirect transmission was in line with those observed in other areas of Spain, including Castile-La Mancha, Valencia, Andalusia, and Zaragoza (12,16–18), while its occupational epidemiological profile was similar to that found in other areas, such as Castile-Leon and Lerida (13,19–24).

Echinococcosis: Spain ranks third among European Union countries for the incidence of echinococcosis, after Bulgaria and Germany (7). This disease is endemic to the Mediterranean area (3), primarily in areas associated with animal husbandry like Extremadura, where this zoonosis has a high socioeconomic impact (25,26). In rural areas it is closely linked to the presence of dogs and sheep that carry the parasite *Echinococcus granulosus*, which may also be borne by wild game (27,28). Based on our data, this disease affected both sexes equally across all age groups, although older individuals tended to be the most affected, probably due to a longer exposure to risk factors, and with a marked predominance in rural areas. We found a slightly lower seroprevalence rate for Extremadura (2.5%) than for other affected Spanish regions, such as Castile-Leon (3.4%) (29) and La Rioja (30). In our study, contact with dogs tended to increase the risk of infection, although this was not statistically significant, probably due to the success of the echinococcosis control program that was implemented in 1983 (31,32) that emphasized pet deworming programs.

Spotted fever: An acute, albeit benign infection with a marked rural prevalence, spotted fever is caused by *Rickettsia conorii* and is transmitted to humans throughout the Mediterranean area due to common dog tick bites (33). We found a seroprevalence rate of 6.4% for Mediterranean fever, which was higher than that observed in the Canary Islands (4.4%) (34) but lower than the rates found in Andalusia (8.7%) and Catalonia (8%) (35,36), with male gender, age, and contact with animals being associated risk factors. This pattern was consistent with the profile associated with this disease (35,37), which in Extremadura may have been related to the number of people who go hunting with dogs and who may occasionally have been bitten by ticks, particularly in the area around Badajoz due to its climatic and orographic conditions (27). Our results showed an increased risk of becoming infected after coming in contact with goats, which indicated that occupational fac-

tors should also be taken into account in the epidemiology of this disease (38).

Leishmaniasis: In Spain, leishmaniasis is caused by *Leishmania infantum*, with urban transmission caused by sand fly bites and with visceral involvement (39). We found a seroprevalence rate of 1.7%, which was similar to that found in the Murcia region (40), but lower than that in Castile-Leon (4.9%) (41). However, unlike these other regions, in Extremadura this infection is more common in urban areas, perhaps because of the growing number of pet-owning families (42), in which both the young and elderly people keep close contact with animals (43). Furthermore, dog culling in countries like Spain is not generally accepted because a dog is considered to be another member of the family. Our study showed that both age and contact with dogs were risk factors for contact with *Leishmania*. Consuming homemade butter also appeared to be significantly associated with a history of contact, although this variable has not been recognized as a risk factor for leishmaniasis.

Toxoplasmosis: This is an urban zoonosis that is caused by *Toxoplasma gondii*. This infection is usually asymptomatic and self-limiting (44), except in immunocompromised patients (45). For humans, the most important sources of infection include contact with cats and eating raw or undercooked meat, contaminated water or food, and via placental transmission (46), as well as contact with soil (47). The seroprevalence rate of this infection in Extremadura at about 35% was moderate and consistent with those found in other countries of central and southern Europe at 30%–50% (48), although prevalence rates vary widely depending on the country (8). In our study, toxoplasmosis incidence was associated with age because the probability of contracting this disease increases with progressive exposures to risk factors, among which consuming pork and pork products is quite significant, particularly in the province of Badajoz that has a tradition of raising and slaughtering pigs at home for family use (49). In our study, no significant differences were found among those individuals who reported contact with cats, despite the known role of these animals in the epidemiology of this disease. Preventing infection is particularly important for non-immunized pregnant women due to the risk of congenital toxoplasmosis (50,51). Among the pregnant women included in our study, 25% had been immunized, which was in accordance with other studies done in Spain (52–54).

Our study had some limitations. The cross-sectional design did not allow us to verify whether or not an infection had occurred recently, so that its association with certain risk factors may have been uncertain. In addition, a possible information bias during data collection may have resulted in observing some unexpected associations. However, despite this possible bias, the classic risk factors for zoonosis were reproduced in our selected sample. Another limitation involves the study population itself. Although we believed that selecting patients who had been to health clinics was an ideal framework with which to conduct a seroepidemiological survey because it promotes patient cooperation and ensures proper data collection, this resulted in another important limitation; the population treated at the health

centers in question may not have adequately reflected the reference population.

Periodic seroprevalence studies provide useful information regarding the evolution of a given population's immunity against the infections studied, which promotes efficient decision-making based on data that represent the actual situations for these infections, and allows for more precisely adjusting the cut-off points for the serological techniques that are used.

In conclusion, despite the continuing decline in the number of zoonosis cases that are reported nationally, human cases are still detected in areas like Extremadura. The most interesting finding of our study was that the observed seroprevalence rate of brucellosis was lower than expected based on the incidence in this region. Also noteworthy was the observed seroprevalence rate of toxoplasmosis, which suggested that more than one third of the population in Extremadura suffered from this infection and that immunized pregnant women were protected from the risk of vertical transmission. The distributions of zoonoses found in this study can be easily associated with the cultural patterns of this region that is characterized by hunting and the raising of livestock, an increase in the number of pets kept by the inhabitants, and the local eating habits associated with raising livestock and other local eating habits. Thus, living in Badajoz was associated with toxoplasmosis primarily through consuming pork products and with spotted fever through hunting. In contrast, in the north of Cáceres, brucellosis was associated with raw-milk cheese consumption.

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Conflict of interest None to declare.

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