



Original Contribution

A SEROLOGICAL SURVEY OF BRUCELLOSIS, ECHINOCOCCOSIS, Q-FEVER, TOXOPLASMOSIS, LEISHMANIASIS AND MEDITERRANEAN SPOTTED FEVER IN ANIMAL PRODUCTION EMPLOYEES IN GREECE

E. Papadogiannakis^{1*}, V. Kontos¹, I. Kontou¹, N. Kostomitsopoulos², E. Siochou¹,
I. Tsachev³, E. Vassalou¹, V. Makropoulos¹

¹National School of Public Health, Athens, Greece,

²Center for Experimental Surgery, Foundation for Biomedical Research,
Academy of Athens, Greece;

³Veterinary Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Objective: To determine the seroprevalence of six zoonoses caused by *Brucella melitensis*, *Echinococcus granulosus*, *Coxiella burnetii*, *Toxoplasma gondii*, *Leishmania infantum* and *Rickettsia conorii* in animal production employees in Greece. **Methods:** 151 blood samples were randomly collected from these employees (Group A), while 122 randomly selected serum samples, kindly provided by blood donation centres from the areas studied, served as controls (Group B). The indirect fluorescence antibody test (IFA) was used for detection of specific IgG antibodies against *Leishmania infantum*, *Rickettsia conorii* and *Toxoplasma gondii* and ELISA for specific IgM and IgG antibodies against *Brucella melitensis*, IgG antibodies against *Echinococcus granulosus* and IgG antibodies against *Coxiella burnetii*. **Results:** Specific antibodies were detected only for brucellosis, Q-fever and toxoplasmosis in both groups. Anti-brucella antibodies were detected in 15 persons of group A (9.9%) and 3 of group B (2.5%). The respective percentages for Q-fever were 21.2% and 13.9%, while for toxoplasmosis 15.2% and 10.7%. However, when comparisons of the results were made for brucellosis there was significant difference between group A and group B ($p < 0.05$). **Conclusion:** Brucellosis could be characterized as occupational disease in employees of animal production sector in Greece.

Key words: Zoonoses, employees, animal production, Greece

INTRODUCTION

As animal husbandry practices and international trade of animals and products of animal origin have been intensified over the last years, the importance of zoonoses is ever increasing for humans. Animal production sector includes breeders, veterinarians, slaughterhouse workers and laboratory technicians and employs a significant number of individuals. Brucellosis, echinococcosis, Q-fever, toxoplasmosis, leishmaniasis and Mediterranean spotted fever are among the most important zoonotic infections, since all can be asymptomatic and transmitted from animals or products of animal origin.

Although certain studies on seroprevalence of certain zoonoses have been conducted in Greece so far, they mainly focused on segments of Greek population not related to animal production sector, the only exception being animal breeders in restricted geographical areas (Spyridaki et al. 1998, Antoniou et al. 2002, Bikas et al. 2003, Avdikou et al. 2005). *Brucella melitensis*, one of the causative agents of brucellosis, is mainly transmitted by the consumption of contaminated dairy products as well as by direct contact with infected animals or animal tissues. *Echinococcus granulosus* causes echinococcosis through direct contact with infested dog or consumption of vegetables derived from contaminated soil, while *Coxiella burnetii*, the causative agent for Q-fever, is transmitted by ticks or inhalation of contaminated aerosols. *Toxoplasma gondii*, an obligate intracellular parasite, is the causative

* **Correspondence to:** Dr. E.I.Papadogiannakis
PhD, National School of Public Health; 196
Alexandras avenue, 115 21 Athens, Greece; E-
mail: papvet@otenet.gr

agent of toxoplasmosis and felids are the only known definitive host of this protozoon (Dubey and Beattie 1988). Visceral leishmaniasis in Greece is caused by *Leishmania infantum*. Dogs are considered the natural reservoir of this protozoon parasite, which is transmitted among canines and humans by bites of infected female sand flies of the genus *Phlebotomus* (Kontos 1986, WHO 1993). *Rickettsia conorii* is the causative agent of the Mediterranean spotted fever and transmitted by infected dog tick *Rhipicephalus sanguineus* (Beran and Steele 1994).

In this study, we aimed to determine the seroprevalence of six zoonoses caused by *Brucella melitensis*, *Echinococcus granulosus*, *Coxiella burnetii*, *Toxoplasma gondii*, *Leishmania infantum* and *Rickettsia*

conorii in employees of the animal production sector in northern, central and southern Greece, using the serological tests, indirect immunofluorescence (IFA) and enzyme linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Study area

The 3 regions of Greece studied in this survey consisted of 4 prefectures, namely Attica, Pella, Thessaloniki and Lassithi. Attica is located in central Greece and basically constitutes the greater Athens area, while Pella and Thessaloniki belong to northern Greece. Lassithi constitutes the eastern part of the island of Crete in southern Greece (**Figure 1**).

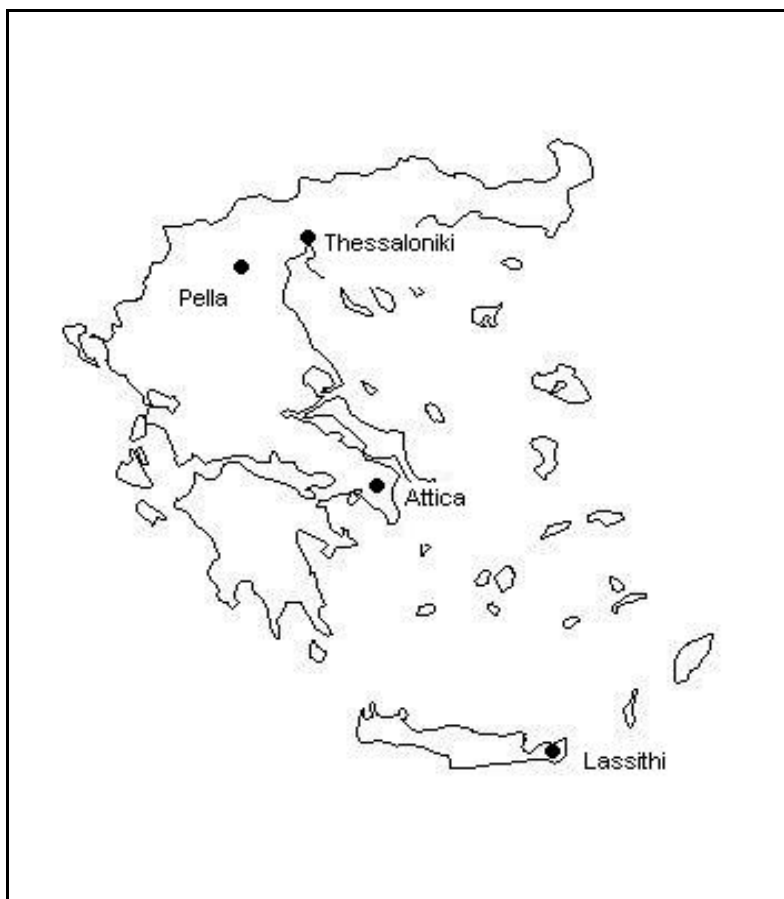


Figure 1: Map of the surveyed areas in Greece

Samples

Consent was obtained from each subject that the serum could be used for the detection of antibodies against several infectious and parasitic agents. In addition, the Ethical Committee of the National School of Public Health approved the study.

A member of the research team filled an already prepared form of questionnaire. Apart

from age and sex, details regarding education level, labour years, occupation, animal species in contact with and disease history related to working environment (included zoonoses), were also recorded. All participants were of Greek nationality, healthy and received no drugs.

A total number of 151 blood samples were randomly collected from the test employees

(Group A). Serum was subsequently separated and stored in Eppendorf tubes at -20°C until

further processing. Additionally, 122 randomly selected serum samples, kindly provided by blood donation centres from the areas studied, served as controls (Group B).

The sampling period, for both Groups, was between September and October 2004.

Serological tests

The indirect fluorescence antibody test (IFA) was used for screening all sera samples for specific IgG antibodies against *Leishmania infantum* (kit Leishmania spot IF, BioMerieux, Lyon, France), *Rickettsia conorii* (kit Panbio limited, Brisbane, Australia) and *Toxoplasma gondii* (kit Antigene Toxo-IF, BioMerieux, Lyon, France). All sera were diluted to 1/50. Positive and negative controls were also tested.

The enzyme linked immunosorbent assay (ELISA) was used for screening all sera samples for specific IgM and IgG antibodies against *Brucella melitensis* (kit Panbio limited, Brisbane, Australia), IgG antibodies against *Echinococcus granulosus* (kit Cypress diagnostics, Langdrop, Belgium) and IgG antibodies against *Coxiella burnetii* (kit Panbio limited, Brisbane, Australia). All sera were diluted to 1/100 according to manufacturer's instructions. Positive and

negative controls were also tested.

Before screening for IgM antibodies against *Brucella melitensis*, sera were absorbed to goat anti-human IgG precipitating antibody preparation in Tris Buffered saline, in order to eliminate competitive inhibition by specific anti-Brucella IgG and remove rheumatoid factor.

Statistical analysis

Biostatistical analysis was performed by the statistical package SPSS, version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Chi-square (χ^2) (Fisher's exact) test was used for the comparison of the prevalence rates between Group A and Group B. The same methodology was used for the comparison of the prevalence rates for the regions studied. The level of 5% was considered significant for all the comparisons made.

RESULTS

Of the 151 participants in the study, 43 (28.5%) were veterinarians, 33 (21.9%) veterinary assistants, 30 (19.9%) breeders, 28 (18.5%) slaughterhouse workers and 17 (11.2%) other personnel related to animal production. The mean duration of occupational activities was 17.23 years (min 1 year – max 55 years) (Table 1).

Table 1: Number of seropositive individuals in group A, in relation to employment years

Employment years	Number of employees *	Brucellosis	Q-fever	Toxoplasmosis
0-5	28	2 (7.1%)	4 (14.3%)	3 (10.7%)
6-10	26	2 (7.7%)	5 (19.2%)	4 (15.4%)
11-15	18	3 (16.7%)	4 (22.2%)	1 (5.6%)
16-20	23	2 (13.0%)	7 (30.4%)	4 (17.4%)
21-25	15	2 (13.3%)	5 (33.3%)	3 (20.0%)
26-30	17	2 (11.8%)	4 (23.5%)	3 (17.6%)
> 30	14	2 (13.0%)	3 (21.4%)	3 (21.4%)

* Only 141 out of 151 subjects stated their employment years

Regarding geographical distribution of subjects in Group A, 47 (31.1%) originated from prefecture of Lassithi (Crete island), 54 (35.8%) from prefecture of Attica (greater Athens area) and 50 (33.1%) from prefectures of Pella and Thessaloniki (northern Greece). 127 (84.1%) were males and 24 (15.9%) females. In Group B, 42 samples (34.4%) were obtained from Lassithi, 39 (31.9%) from Attica and 41 (33.6%) from Pella and

Thessaloniki.

149 individuals in Group A stated their educational level. 62 (41.6%) had completed a primary and 35 (23.5%) a secondary education school while 52 (34.9%) were university graduates. The profession of 140 out 151 participants was related to direct exposure to various animal species (Table 2).

Table 2: Animal species, which the participants in the study were in contact with.

<i>Animal species *</i>	<i>Number of employees</i>
a, b and c	37 (24.5%)
a, b, c, d and e	21 (13.9%)
b	27 (17.9%)
d	22 (14.6%)
a	8 (5.3%)
c	3 (2.0%)
a and b	9 (6.0%)
b and d	5 (3.3%)
b, c and d	4 (2.6%)
d and e	1 (0.7%)
b and c	1 (0.7%)
b, c and e	1 (0.7%)
a, b, c and d	1 (0.7%)

* a: Bovine

b: Small Ruminants

c: Swine

d: Companion Animals

e: Poultry

70 individuals of Group A, stated that they had been affected by a disease related to the environment of their profession. In particular, 35 (23.2%) referred injuries, 7 (4.6%) allergies, 9 (6.0%) zoonoses, 4 (2.6%) miscellaneous, 4 (2.6%) injuries along with allergies, 5 (3.3%) injuries and zoonoses, 2 (1.3%) allergies and zoonoses, 2 (1.3%) injuries, allergies and zoonoses, 1 (0.7%) zoonoses and miscellaneous and finally 1 (0.7%) injuries, allergies and miscellaneous.

21 individuals stated they had been affected by zoonoses. In particular, 18 (11.9%) had a history of brucellosis (*Brucella melitensis*), 1 (0.7%) of echinococcosis and 2 (1.3%) of toxoplasmosis. However, all individuals were clinically normal and felt quite well the day of consultation and blood sampling.

As far as brucellosis was concerned, anti-*Brucella* IgG antibodies were detected in 15 (9.9%) individuals of Group A. 9 (19.1%) seropositive people were detected in Crete, 2 (3.7%) in Attica and 4(8%) in northern Greece. As regards the educational level 9 (14.5%) positive individuals were found among the employees with a low level, 1(2.9%) among those with a modest and 5(9.6%) among those with a higher educational level. In Group B, 1 (2.5%) sample from Attica was positive to *Brucella* IgG antibodies, 2 (4.8%) from northern Greece and none from Crete. Anti-brucella IgM examinations were negative in both groups. Comparison between groups A and B,

as far as the positive results are concerned, showed a significant difference over the former ($p=0.014$). Only for Crete region was a significant difference found between seropositive individuals - originated from this area - between the two groups ($p= 0.03$).

Anti-*Coxiella burnetii* IgG antibodies were found in 32(21.2%) subjects of Group A. 19(40.4 %) seropositive individuals were detected in Crete, 6(11.1%) in Attica and 7(14%) in northern Greece. 12 out of 62 individuals with low educational level were found seropositive to *C. burnetii*. The incidence of Q-fever among modest and higher educational level people was 9 (25.7%) and 10 (19.2%), respectively. In Group B, 2 (5.1%) samples from Attica were positive to *Coxiella burnetii* IgG antibodies, 11 (26.8%) from northern Greece and 4 (9.5%) from Crete. Comparison between groups A and B, as far as the positive results are concerned, showed no significant difference over the former ($p=0.153$). Only for Crete region was a significant difference found between seropositive individuals - originated from this area - between the two groups ($p= 0.01$).

Anti-*Toxoplasma gondii* IgG antibodies were found in 23 (15.2%) individuals in Group A. 16 (34%) seropositive subjects were detected in Crete, 1 (1.8%) in Attica and 6 (12%) in northern Greece. 11(17.7%), 7(20%) and 3(5.8%) was the incidence of seropositive individuals among low, modest and higher educational level persons respectively. In Group B, 1 (2.5%) sample from Attica was

positive to *Toxoplasma gondii* IgG antibodies, 6 (14.6%) from northern Greece and 6 (14.2%) from Crete. Comparison between groups A and B, as far as the positive results were concerned, showed no significant difference over the former ($p=0.286$). Only for Crete region was a significant difference found between seropositive individuals - originated from this area - between the two groups ($p=0.048$).

Of all sera samples examined, in both Group A and Group B, none found positive for antibodies against *Echinococcus granulosus*, *Leishmania infantum* and *Rickettsia conorii*.

From the seropositive subjects 15 (34.9%) were veterinarians, 17 (51.5%) vet. assistants, 17 (56.6%) breeders, 14 (50%) slaughterhouse workers and 6 (54.5%) other personnel related to animal production (Table 4).

The number of seropositive individuals of Group A in relation to animal species to which they were in contact with is shown on

Table 3.

DISCUSSION

This study confirmed that exposure to *Brucella melitensis* remains an occupational hazard among people employed by the animal production sector in Greece, including breeders, veterinarians, laboratory staff and slaughterhouse workers. Professional occupation with animals was also found to be a risk factor for human brucellosis in a study conducted in a rural area of Achaia in north-western Peloponnese in Greece (Bikas et al. 2003). Previous studies have demonstrated that the incidence of brucellosis caused by *Brucella melitensis* in sheep, goats and humans is a very significant problem in Greece (Taleski et al. 2002) whereas in two villages in Crete human brucellosis was not common in contrast to Q-fever, toxoplasmosis, echinococcosis and Mediterranean spotted fever (Antoniou et al. 1995).

Table 3: Number of seropositive individuals of Group A in relation to animal species to which they were in contact with.

<i>Animal species</i> *	<i>Number of employees</i>	<i>Brucella +</i>	<i>Q-fever</i> +	<i>Toxoplasmosis</i> +
a, b and c	37 (24.5%)	3	8	9
a, b, c, d and e	21 (13.9%)	3	6	6
b	27 (17.9%)	5	5	2
d	22 (14.6%)	0	2	0
a	8 (5.3%)	1	2	0
c	3 (2.0%)	0	0	0
a and b	9 (6.0%)	1	3	0
b and d	5 (3.3%)	0	1	3
b, c and d	4 (2.6%)	1	1	1
d and e	1 (0.7%)	0	0	0
b and c	1 (0.7%)	0	1	0
b, c and e	1 (0.7%)	0	0	1
a, b, c and d	1 (0.7%)	0	1	0

* a: Bovine

b: Small Ruminants

c: Swine

d: Companion Animals

e: Poultry

Table 4: Number of seropositive persons of Group A in relation to their profession

<i>Profession</i>	<i>Number of employees</i>	<i>Brucellosis +</i>	<i>Q-fever</i> +	<i>Toxoplasmosis</i> +
Veterinarians	43	4	9	2
Vet. assistants	33	5	8	4
Breeders	30	4	6	7
Slaughterhouse workers	28	1	7	6
Other personnel	17	1	2	3

Lassithi prefecture (Crete, southern Greece) had the higher incidence of brucellosis among

the subjects of Group A (19.1%) which was significant ($P=0.03$). In Crete, an average of

20% sheep was found to be seropositive to *Brucella melitensis* (Stephanakis and Mpizaki 1993). It has been shown that the prevalence of brucellosis in sheep and goats correlates with the incidence of the disease in humans (Minas et al. 2004). Accordingly, an incomplete application of control and eradication program in small ruminants in Crete, including mass vaccination program of young and adult animals with Rev-1 vaccine, could be the reason for the persistence of brucellosis in subjects of Group A in Lassithi region. Our results showed that 8% of the individuals of Group A from northern Greece were seropositive to *Brucella melitensis*, while the respective incidence in Attica was 3.7%. Since the prefectures studied in northern Greece are mainly rural in contrast to Attica area, which is urban in large part, such disease incidence differences in these areas could be partially explained.

The presence of specific anti-*Brucella* IgM in serum probably indicates a relatively recent primary infection either symptomatic or subclinical (Gilbert and Hawes 1981). Since no individual in Group A was found to be positive for *Brucella* IgM antibodies and the test used were highly specific, a recent exposure to *Brucella melitensis* could not be confirmed. In contrast, chronic, asymptomatic or subclinical infection usually occurs among individuals of this segment of the population, because a significant number of *Brucella* IgG antibodies were detected. Repeated or prolonged exposure is associated with *Brucella* IgG antibodies, irrespective of symptoms (Gilbert and Hawes 1981, Corbel 1997). Since brucellosis is an intracellular infection, the proper activation of cellular immunity can either eliminate or isolate the bacterium while at the same time down-regulate the humoral immune response and the amount of specific antibodies produced. It is therefore likely that in either group a certain, probably low number of individuals, could be exposed to *Brucella* organisms without specific antibodies be detected. Thus, a probably low percentage of false negative results have to be taken into account. On the other hand, it is well known that people employed in the animal production sector in general, also have a high risk of exposure to other Gram-negative bacteria, like *Yersinia enterocolitica* 09, *Francisella tularensis* and *E.Coli* 0157 which are known to cross-react with antisera against *Brucella* (Corbel 1985). Because these mentioned bacteria usually cause symptomatic diseases and to a lesser extent subclinical or asymptomatic ones and

the specificity of the test used was 97%, false positive reactions seem to be highly unlikely. In general, seroprevalence studies may be criticized in that they cannot distinguish between unimportant exposure and clinically significant disease. Nevertheless, the significant difference found in Group A over Group B, as regards the positive results for brucellosis, confirms that this disease remains a definite risk in employees of the animal production sector in Greece (mainly in Crete) and suitable prevention is warranted until full eradication has been achieved. Until then, a combination of appropriate public health policy and the implementation of safe work practices will remain the most successful approach to decreasing the incidence of brucellosis.

Farm animals, mainly cattle, sheep and goats, along with pets are considered the primary reservoirs of *Coxiella burnetii*. High numbers of this bacterium are present in the placenta of infected parturient animals and are shed in the environment following labour or abortion. Humans usually acquire the infection mainly by inhalation of contaminated aerosols or ingestion of unpasteurised dairy products (Maurin and Raoult 1999). Although Q-fever is usually directly associated with contact with infected animals and their products, the seroprevalence comparison between groups A and B was found to be not significant. However, again, Lassithi prefecture was found with the higher incidence of Q-fever among the subjects of Group A (40.4%), which was significant ($p=0.01$). Probably, the contact with animals seems not to be the main route of transmission of *Coxiella burnetii* in Greece, the exception being Crete. It would be interesting, the incidence of seroprevalence of *Coxiella burnetii* to be studied in animals in Crete. Nowadays, there is an increasing number of Q-fever cases among urban residents, which may be attributed to the increase exposure opportunities to animals, through travel to the countryside but also to urbanization of rural areas (Hellenbrand et al. 2001). Additionally, although pasteurised dairy products are usually considered safe, in a recent outbreak of Q-fever in Newfoundland, Canada, consumption of cheese made from pasteurised milk emerged as a risk factor for infection for the first time (Hatchette et al. 2001). The above and possibly other factors are likely to be much more prevalent for Q-fever transmission in Attica and northern Greece, than Crete.

Toxoplasmosis is one of the most

frequent parasitic infections with a worldwide distribution. Although the number of seropositive subjects of Group A was higher than that of Group B, it was not significant. This is not surprising since although domestic and free living animals are the reservoirs of *Toxoplasma gondii*, with cats, pigs, sheep, cattle being the most important species, infection mainly result from consumption of undercooked or raw meat containing parasitic cysts, or from contact with food, water or sand contaminated with oocysts spread by infected cats, while laboratory staff can be infected by handling infective material (Dubey and Beattie 1988). Lassithi prefecture was found with the higher incidence of toxoplasmosis among the subjects of Group A (34%), which was marginally significant ($p=0.048$). Accordingly, in Crete, contact with animals seems to be the primary reason for transmission of the disease in this region. In both Groups, Crete was found with the higher incidence of seropositive subjects followed by northern Greece. If it is taken into account that Greeks are not usually fans of undercooked meat, it is reasonable to consider that other routes of transmission of *Toxoplasma gondii* may play also a role, such as handling of raw meat, gardening with naked hands and indirect transmission by wild rodents. The large number of stray cats and wild rodents in these regions could explain the results, at least in part. The results of Group B in our study are in agreement with those of other similar studies conducted in northern Greece (Diza et al. 2005) and Crete (Antoniou et al. 2004).

Although toxoplasmosis is usually an asymptomatic infection, in cases of pregnant women and immunosuppressed individuals, the parasite can be reactivated and cause severe disease either in the foetus or in the infected person itself. Accordingly, public health authorities should keep the public informed about the preventive measures for toxoplasmosis, in particular in Crete.

The higher incidence of brucellosis, Q-fever and toxoplasmosis was recorded among employees with many years in the profession (Table 1). Although the inexperience and enthusiasm, early in the course of professional carrier, have been implicated as factors contributing to the infection by *Brucella melitensis* the results of this study do not support such an aspect.

The higher incidence of brucellosis, toxoplasmosis and Q-fever was recorded among veterinary assistants, breeders and slaughter house workers, respectively (Table

4).

The degree of antibody response to the echinococcus cyst is mediated by its location, the degree of calcification and mainly the immune response of the individual to the parasite. Usually, liver cysts produce a higher antibody response than lung cysts. The test used in our study for detection of antibodies to *Echinococcus granulosus* has a high sensitivity for parasite antigens, the possibility of false negative results is highly unlikely (Coltorti 1986). It has been shown that ELISA serology may find “abortive” alveolar echinococcosis lesions and early cases before the development of typical images (Bartholomot et al. 2002). Both Groups in our study were found negative for echinococcosis. Since the dog is the natural reservoir of the adult tapeworm and humans and other animals are infested by the ingestion of parasite ova, it is reasonable for one to suggest that dogs in Greece, in large part, are free of *Echinococcus granulosus*. However, the later assumption seems not to be the case, since several studies conducted in Greece have demonstrated high incidence of hydatidosis in food animals (Himonas et al. 1994, Theodoropoulos et al. 2002, Sotiraki et al. 2003). Accordingly, the implementation of proper preventive measures seems to play a major role here, a fact points towards a good and effective veterinary profession in Greece over the last 10-15 years, since echinococcosis constituted a significant public health problem 20 or 30 years ago.

Despite Greece is an endemic country for leishmaniasis (*Leishmania infantum*), we found no seropositive individuals in either group using the highly sensitive method of IFA. *Leishmania infantum* is an intracellular protozoon and proper activation of cellular immunity can either eliminate or isolate the parasite while at the same time down regulates the humoral immune response and the amount of specific antibodies produced. It is likely that a certain, probably a low number of individuals examined, could have been exposed to leishmania parasites without specific antibodies to be detected. Although false negative reactions have to be taken into account, Greek National Centre of Disease Control and Prevention as well as other studies refer only a small number of leishmaniosis cases per year (Papadopoulou et al. 2005). On the other hand, in comparison to other Mediterranean countries, Greece appears to have high background of canine leishmaniasis, second only to Sicily (Papadopoulou et al. 2005). If it is also taken into account the high number of stray dogs in

Greece, a possible explanation of our negative results could be the effective implementation of antiphlebotome (*Phlebotomus* sandflies transmit the disease) repellents and effective activation of cellular immunity of the individuals, since sand fly tropism seems not to occur (Killick-Kendrick and Killick-Kendrick 1999).

The Mediterranean spotted fever seems to be rare either among employees of the animal production sector or in general population since we found no seropositive individuals in both groups. Although it has been claimed that IFA presents the lower cross reactions between *Rickettsia conorii* and other *Rickettsia* species (Newhouse et al. 1979) the results of our study show that *Rickettsia* infections seem to be uncommon. In a study conducted among 1584 randomly selected people from prefecture hospitals in northern Greece, *Rickettsia conorii* antibodies were detected in 125 persons (7.9%) (Alexiou-Daniel et al. 2002). However, according to the same study, the percentage of antibodies to *Rickettsia conorii* varies significantly among prefectures in Greece with those we studied to demonstrate a very low percentage. This could explain the different findings of our study. Another study, conducted in three rural villages in the prefecture of Fokida in central Greece, demonstrated a high incidence (about 50%) of seropositive individuals to *Rickettsia conorii* (Babalís et al. 1993). Some environmental or other factors related to tick (vector of the *Rickettsia conorii*) biology could explain these differences among prefectures in Greece, since *Rickettsia conorii* has been isolated from ticks in central Greece (Psaroulaki et al. 2003). A tick bite is likely to occur rarely in order for the low incidence of the disease to be explained in Greece.

Acknowledgement:

The authors would like to gratefully thank the Hellenic Institute for Health and Safety at Work (ELINYAE) for funding this study, and D. Zavras for statistical analysis.

REFERENCES

- Alexiou-Daniel S., Manika K., Arvanitidou m. and Antoniadis A. (2002) Presence of *Rickettsia conorii* and *Rickettsia typhi* infection in the population of northern Greece. Am J Trop Med Hyg, 66(1):76-79.
- Antoniou M., Tselentis Y., Babalis T., Gikas A., Stratigakis N., Vlachonikolis I.,

Kafatos A. and Fioretos M. (1995) The seroprevalence of ten zoonoses in two villages of Crete, Greece. Eur J Epidemiol, 11(4):415-423.

- Antoniou M., Tzouvali H., Sifakis S., Galanakis E., Georgopoulou E., Liakou V., Giannakopoulou C., Koumantakis E. and Tselentis Y. (2004) Incidence of toxoplasmosis in 5532 pregnant women in Crete, Greece: management of 185 cases at risk. Eur J Obstet Gynecol Reprod Biol, 117(2):138-143.
- Antoniou M., Economou I., Wang X., Psaroulaki A., Spyridaki I., Papadopoulos B., Christidou A., Tsafantakis E. and Tselentis Y. (2002) Fourteen -year seroepidemiological study of zoonoses in a Greek village. Am J Trop Med Hyg, 66(1):80-85.
- Avdikou I., Maipa V. and Alamanos Y. (2005) Epidemiology of human brucellosis in a defined area of northwestern Greece. Epidemiol Infect, 133(5):905-910.
- Babalis T., Dupont H.T., Tselentis Y., Chatzichristodoulou C. and Raoult D. (1993) *Rickettsia conorii* in Greece: comparison of a microimmunofluorescence assay and western blotting for seroepidemiology. Am J Trop Med Hyg, 48(6):784-792.
- Bartholomot B., Vuitton D., Harraga S., Shi D., Giraudoux P., Barnish G., Wang Y., Macpherson C. and Craig P. (2002) Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. Am J Trop Med Hyg, 66(1):23-29.
- Beran GW and Steele JH. (1994) Handbook of Zoonoses. Section A: Bacterial, Rickettsial, Chlamydial and Mycotic, Second Edition, CRC Press, New York..
- Bikas C., Jelastopulu E., Leotsinidis M. and Kondakis X. (2003) Epidemiology of human brucellosis in a rural area of north-western Peloponnese in Greece. Eur J Epidemiol, 18(3):267-274.
- Coltorti EA. (1986) Standardization and evaluation of an enzyme immunoassay as a screening test for the seroepidemiology of human hydatidosis. AM J Trop Med Hyg 35(5): 1000-1005.
- Corbel M.J. (1997) Brucellosis: an overview. Emerg Infect Dis, 3:213-221.
- Corbell JM. (1985) Recent advances in the study of brucella antigens and their

- serological cross-reactions. Vet Bull. 55: 927-942.
- Diza F., Frantzidou F., Souliou E., Arvanitidou M., Gioula G. and Antoniadis A. (2005) Seroprevalence of *Toxoplasma gondii* in northern Greece during the last 20 years. Clin Microbiol Infect, 11(9):719-723.
 - Dubey J.P. and Beattie C.P. (1988) Toxoplasmosis of animals and man. CRC Press Inc. Boca Raton.
 - Gilbert GL and Hawes LA. (1981) The antibody response to *Brucella*: immunoglobulin response measured by enzyme-linked immunosorbent assay and conventional tests. Aust N Z J Med 11(1): 40-45.
 - Hatchette T.F., Hudson R.C., Schlech W.F., Campell N.A., Hatchette J.E., Ratman S., Raoult D., Donovan C. and Marrie T.J. (2001) Goat associated Q fever: a new disease in Newfoundland. Emerg Infect Dis, 7:413-419.
 - Hellenbrand W., Breuer T. and Petersen L. (2001) Changing epidemiology of Q fever in Germany 1947-1999. Emerg Infect Dis, 7:789-796.
 - Himonas C., Antoniadou-Sotiriadou K. and Papadopoulos E. (1994) Hydatidosis of food animals in Greece: prevalence of cysts containing viable protoscoleces. J Helminthol, 68(4):311-313.
 - Killick-Kendrick R. and Killick-Kendrick M. (1999) Biology of sand fly vectors of Mediterranean canine leishmaniasis. Canine Leishmaniasis an update. Proceedings of the International Canine Leishmaniasis Forum, Barcelona, Spain, 26-31.
 - Kontos V. I. (1986) A contribution to the study of canine leishmaniasis. PhD thesis, Veterinary Faculty, Aristotle University of Thessaloniki.
 - Maurin M. and Raoult D. (1999) Q fever. Clin Microbiol Rev, 12:518-553.
 - Minas A., Minas M., Stournara A. and Tselepidis S. (2004) The "effects" of Rev-1 vaccination of sheep and goats on human brucellosis in Greece. Prev Vet Med, 64(1):41-47.
 - Newhouse V.F., Shepard C.C., Redus M.D., Tzianabos T. and McDade J.E. (1979) A comparison of the complement fixation, indirect fluorescent antibody and microagglutination tests for the serological diagnosis of rickettsial diseases. Am J Trop Med Hyg, 28:387-395.
 - Papadopoulou C., Kostoula A., Dimitriou D., Panagiou A., Bobojianni C. and Antoniadis G. (2005) Human and canine leishmaniasis in asymptomatic and symptomatic population in northwestern Greece. Journal of Infection, 50:53-60.
 - Psaroulaki A., Spyridaki I., Ioannidis A., Babalis T., Gikas A. and Tselentis Y. (2003) First isolation and identification of *Rickettsia conorii* from ticks collected in the region of Fokida in central Greece. Journal of Clinical Microbiology 41(7): 3317-319.
 - Reid A. J. (2005) Brucellosis - A persistent occupational hazard in Ireland, 11:302-304.
 - Sotiraki S., Himonas C. and Korkoliakou P. (2003) Hydatidosis-echinococcosis in Greece. Acta Trop, 85(2):197-201.
 - Spyridaki I., Gikas A., Kofteridis D., Psaroulaki A. and Tselentis Y. (1998) Q fever in the Greek island of Crete: Detection, isolation and molecular identification of eight strains of *Coxiella burnetii* from clinical samples. J Clin Microbiol, 36(7):2063-2067.
 - Stefanakis A. and Mpizaki A. (1993) A study of toxoplasmosis in sheep and goats in Crete. Geotechnical Chamber of Greece, Cretan Branch, 164-222.
 - Taleski V., Zerva L., Kantardjiev T., Cventic Z., Erski-Biljic M., Nikolovski B., Bosnjakovski J., Katalinic-Jankovic V., Panteliadou A., Stojkoski S. and Kirandziski T. (2002) An overview of the epidemiology and epizootology of brucellosis in selected countries of central and southeast Europe. Vet Microbiol 90(1-4):147-155.
 - Theodoropoulos G., Theodoropoulou E., Petrakos G., Kantzoura V. and Kostopoulos J. (2002) Abattoir condemnation due to parasitic infections and its economic implications in the region of Trikala, Greece. J Vet Med B Infect Dis Vet Public Health, 49(6):281-284.
 - WHO. (1993) Epidemiology, diagnosis and control of leishmaniasis in the Mediterranean area MZCP/LEISH/93.3 Athens, Greece.