The seroprevalence of ten zoonoses in two villages of Crete, Greece

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Abstract. The seroprevalence and incidence of 10 zoonoses due to Rickettsia typhi, R. conorii, Coxiella burnetii, Burcella sp., Borrelia sp., Toxoplasma sp., Entamoeba histolytica, Echinococcus granulosus and Fasciola hepatica were studied in an animal husbandry and a farming village in Crete, Greece. The serum conversion incidence of each infectious agent was determined by testing 2 blood samples, collected in 1985 and in 1987. The surveillance was conducted using detailed transparent maps of the 2 villages studied, on which epidemiological data were interrelated to the results obtained from the serological tests. Thus the importance and spread of each infection were visualized. C. burnetii, Toxoplasma sp., R. conorii, and E. granulosus, were the most common infectious agents encountered during this study.

Key words: Coxiella, Rickettsia, Toxoplasma, Zoonoses

Introduction

Zoonoses constitute a primary health hazard for people living in the countryside, especially in areas with a temperate climate. Yet, their importance has been underestimated. The purpose of this study was to collect data on the seroprevalence and incidence rate of the most common zoonoses, in 2 villages in Crete, using mapping in the surveillance. Such data would be useful in designing the primary health care services, so as to facilitate for the rapid diagnosis of such zoonoses, and help in their eradication.

Ten infectious agents of bacterial, rickettsial and parasitological origin were studied. They were: Rickettsia typhi, responsible for murine typhus that is known to occur in all continents. It is transmitted by the rat flea Xenopsylla cheopis; R. conorii, causative agent of boutonneuse fever, which in the Mediterranean area is transmitted by the dog tick Rhipicephalus sanguineus [13]; Coxiella burnetii, the infectious agent of Q fever, with worldwide distribution [29]; Burcella sp., the pathogen responsible for Brucellosis; Borrelia sp., the causative agent of lymph disease that is transmitted by Ixodes dammini and other related ticks [38]. It is found in the USA and parts of Europe; Toxoplasma gondii, an obligate intracellular protozoan which causes human toxoplasmosis with worldwide distribution; Leishmania spp., which are transmitted by sandflies and cause leishmaniasis; Entamoeba histolytica, a unicellular parasite responsible for amoebiasis in the tropics and in temperate regions; Echinococcus granulosus, a tapeworm, which persists in nature by infecting dogs, sheep and cattle and accidentally humans; finally, the liver fluke Fasciola hepatica, causative agent of a cosmopolitan zoonosis called fascioliasis. There have been no previous population-based surveys for most of these infectious diseases in Crete.

Materials and methods

Serum collection. People living in 2 villages in the island of Crete (Southern Greece), made up the target population. The 2 villages chosen for this study were: (1) Tymbaki, 4,000 inhabitants, Iraklion prefecture, at 25 m altitude, where people are almost exclusively farmers (greenhouse agriculture mainly); (2) Anogia, 2,500 inhabitants, Rethymnon prefecture, at 750–800 m altitude, where people work almost exclusively in animal husbandry (rearing of sheep, goats and swine). Sampling was conducted in 2 periods: 1985 and 1987. The people in each village were informed about the purpose of the study and were very helpful and willing to participate in the project. The sample was selected randomly and consisted of 97 households (Table 1). All members of each participated household were examined, 419 persons in all. Serum was obtained from each individual in the sample population, once for each sampling period. It was stored at -20 °C until the time of the assay. A questionnaire was prepared in order to collect all relevant epidemiological information about the participants. Persons over 18 years of age were considered adults.
Table 1. The sample used in the epidemiological study and the seroprevalence of the ten zoonoses studied during the 2 sampling periods in the 2 villages

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>R. typhi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R. conorii</td>
<td>13</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>c. burnetii</td>
<td>80</td>
<td>8</td>
<td>11 15 114</td>
</tr>
<tr>
<td>Brucella sp.</td>
<td>0</td>
<td>3</td>
<td>1 0 4</td>
</tr>
<tr>
<td>Borrelia sp.</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Toxoplasma sp.</td>
<td>62</td>
<td>15</td>
<td>71 14 162</td>
</tr>
<tr>
<td>Leishmania spp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. histolytica</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E. granulosus</td>
<td>0</td>
<td>1</td>
<td>0 0 1</td>
</tr>
<tr>
<td>F. hepatica</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NC, New cases.

Assays. All assays were performed using coded specimens without clinical or other information. Sera collected were examined for:

1. Antibodies IgG, IgM and IgA against R. typhi, R. conorii and Coxiella burnetii using the indirect immunofluorescence (BioMerieux, France), in 1/25 (IgM, IgA) and 1/60 (IgG) original dilutions and respective dilutions (positive titers were considered > 1/60). All conjugates used were provided by Diagnostics Pasteur, France.

2. Antibodies against Brucella sp. using 2 methods: (a) the Rose-Bengal card test (positive titers >1/80) (Brucelloslide, BioMerieux, France); (b) indirect immunofluorescence (cutoff titers >1/20).

3. Antibodies against Borrelia using the indirect immunofluorescence, in original dilution of 1/20 and respective dilutions (positive titers >1/128).

4. Antibodies against Toxoplasma using 2 methods: (a) latex (Labo Fumouze, France); (b) indirect immunofluorescence (Diagnostics Pasteur, France) in 1/50 original dilutions and respective dilutions (Positive titers > 1/128).

5. Two methods were employed for the detection of Amoeba, Echinococcus and Fasciola antibodies: (a) the haemaggulination assay (BioMerieux and Labo Fumouze, France), and (b) the counter-immunoelectrophoresis (CIE), using cellulose acetate strips. Method 'a' was performed first, in 1/60 original dilutions. Sera positive in >1/200 dilutions, were further tested with methods 'a' and 'b' (positive titers for haemaggulination >1/400 for Amoeba, and >1/800 for the other 2). The criteria used in CIE, was the existence of the specific arc for each pathogen. The CIE and IF were used for the detection of Leishmania.

The antigens used in CIE for Leishmania, Echinococcus and Fasciola were prepared in our laboratory [24].

In cases of positive serological tests regarding E. granulosus and C. burnetii, the people concerned were called in for further medical examinations and when possible, appropriate treatment was administered.

Mapping. Aerial photographs and detailed maps of the 2 villages, were obtained from the Ministry of the Environment. In addition, 7 new maps for each village were constructed on transparent paper so that the maps could be superimposed on each other for relating information. The new maps (scale 1:1000) contained the following information.

Map 1. The village and the surrounding area were divided into neighborhoods and each house, shop, greenhouse etc. was given a code number. This code number had 2 parts. The first part indicated the neighborhood and the second part, the house. Additionally, the maps were divided into 3 main areas each: a, b, c, depending on the presence of dirt or paved secondary roads, the existence of fields in the area and other such characteristics. Anogia for example, is built in 2 altitudes: 800 m (area a and b) and 750 m (area c).

Map 2. Schools, playgrounds, parks.

Map 3. Places where people gather together, for example, churches, old people's homes, coffee shops, taverns, etc.

Map 4. State services, cheese making factory, farming co-op.

Map 5. Abattoir, stables, yards near houses with animals, abattoir's dumping round, greenhouses, fields, chicken houses, threshing-floors.

Map 6. Hotels, rooms for rent, squares, shops, health center.

Map 7. Water supply network, drainage network, wells, torrents, cisterns.

In addition, each map had marks which aided in superimposing the maps accordingly. Different colors were used to mark the information on the maps. New maps on transparent paper were constructed, containing the serological data obtained for each infection. This way, areas where accumulated cases of people that had come in contact with an infectious agent, were identified. These maps were then compared to the maps with the epidemiological information, in an attempt to trace the source of the infection.

Parallel to this work, Phlebotomus spp. were col-