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ORIGINAL ARTICLE

THE USE OF IMMUNOENZYMATIC METHOD FOR DETECTION OF ANTIBODIES AGAINST ZOONOTIC DISEASES IN CZECH SOLDIERS RETURNING FROM AFGHANISTAN

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Summary
In this work, we focused on detection of IgG antibodies in the blood sera from Czech soldiers returning from Afghanistan against selected zoonotic diseases by commercially available ELISA kits. Samples were tested for the presence of antibodies against *Brucella abortus*, *Coxiella burnetii*, *Leptospira* spp., complex of *Rickettsia conorii*, *Leishmania infantum*, hantaviruses and hepatitis E virus. Except for *L. infantum* (all persons were negative), we found following seroreactivity rate: 10 % in *B. abortus*, 11 % in *C. burnetii*, 20 % in *Leptospira* spp., 10 % in *R. conorii* and 4 % in both hepatitis E virus and hantaviruses.

Key words: ELISA method; zoonoses; soldiers; Afghanistan

INTRODUCTION

The zoonoses are infectious diseases that are transmitted to humans from animals. Their agents can be parasites, fungi, bacteria, viruses or prions [1]. We selected seven zoonotic agents that should be tested in soldiers returning from missions – *Brucella*, *Leishmania*, *Rickettsia*, *Leptospira*, *Coxiella*, hepatitis E virus, and hantavirus [2]. Afghanistan lies in several climate zones in the south-west Asia. Its surface is very ragged and hostile. Years of wars and civil disorders led to huge flood of soldiers, peacekeepers, humanitarian workers and journalists. Also the number of refugees and internally displaced persons increased, which, with the number of foreigners, enhanced the epidemiological risk, including zoonoses. The population in Afghanistan is affected by many infectious diseases – especially diarrhea, respiratory infections and measles [3]. Soldiers go to tropical or subtropical places with low hygiene standards and with the presence of many infectious diseases [4]. Despite of having clean water and food, clean place to stay and being vaccinated, the soldiers should take into account the risk of infection.

Bacteria of the genus *Brucella* are gram negative coccobacilli, causing a worldwide known disease called brucellosis. It affects primarily animals but can easily be transmitted to humans. The most dangerous species are *Brucella melitensis*, *B. abortus*, *B. suis*

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and *B. canis* [5]. The reservoir of these bacteria can be cattle, goats, sheep, camels, pigs and some other animals. People can be infected by direct contact with animals, infected dust, contaminated milk or dairy products [6].

Coxiella belongs to gram negative and intracellular bacteria. *Coxiella burnetii* is the only member of this genus and it causes Q fever. It occurs worldwide and it is dangerous for both people and animals. People can get infected in the same ways as with *Brucella* and additionally by ticks [1].

Leptospira belongs to class *Spirochaetes* and it is gram negative. It causes disease called leptospirosis. Its hosts and reservoirs are rodents, insectivores, dogs, cattle, pigs, horses and many more. The most frequent way of infection is contact with contaminated water, soil or food [7].

Bacteria from the genus *Rickettsia* are small gram negative and intracellular organisms. They are agents of several diseases. They are associated with haematophagous arthropods (ticks, fleas, lice, chiggers) and some of them are reservoir and vectors of the disease. In the Middle East, only *Rickettsia conorii* occurs. It can cause Mediterranean fever and other fevers. In addition to high fever, the disease is characterized by rash and eschar [8].

Leishmania is a protozoon causing leishmaniasis that occurs endemically in tropical and subtropical regions. It is transmitted by phlebotomus. *Leishmania* species differ in disease symptoms and geographic distribution. Some of them cause cutaneous, mucocutaneous or visceral leishmaniasis.

Hantaviruses belong to the family *Bunyaviridae* and they occur in Asia, America, Africa and Europe. They cause two main types of disease: haemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS). Their natural host and reservoir are rodents [1].

Hepatitis E virus is non-enveloped virus and it is the only member of the genus *Hepevirus*. The five genotypes are known and four of them occur in people [8]. The transmission is faecal-oral through contaminated water or food, but there is also a possibility of zoonotic transmission.

MATERIALS AND METHODS

Blood samples

For this study, the blood sera were collected from 70 asymptomatic persons staying in Afghanistan in 2011 after their arrival and returning to the Czech Republic and were supplied from Czech Army Serum Bank. The blood samples were centrifuged in the laboratory 3 to 6 hours after collection and the separated sera were stored at -20 ºC.

ELISA assay

We used commercially available ELISA kits for determination of IgG antibodies (Table 1). These kits apply indirect ELISA method. Every test was performed according to manual provided by the producer.

After measurement of optical density (OD) by spectrophotometer, we calculated results by using formula provided in manual. These results are also interpreted according to a table found in each brochure, most often as a ratio of sample OD and the cut-off value.

Table 1. ELISA kits used in our testing.

Zoonotic agents	ELISA kit
Brucella abortus	Brucella-IgG, NovaTec Immunodiagnostica
Coxiella burnetii	Coxiella-IgG, DIAMedix, Delta Biologicals
Leptospira spp.	SERION ELISA *classic* IgG leptospira
Rickettsia conorii	Rickettsia-IgG, Fuller laboratories
Leishmania infantum	Leishmania-IgG, NovaTec Immunodiagnostica
Hantavirus Eurasia (Hantaan, Dobrava, Puumala)	Anti-Hanta virus pool 1 Eurasia, EUROIMMUN
Hepevirus	HEV IgG, DIA.PRO

RESULTS AND DISCUSSION

The results are given in Table 2.

Brucella abortus – out of 70 tested sera, 7 sera were positive (10 %) for the presence of IgG antibodies against *B. abortus* W 99. We can assume that if there was an infection, it was mild or inapparent. Unfortunately, the producer doesn´t provide an exact information about what antigen was used. In many cases, the immunotests mainly contain proteins of outer membrane. The most cases of human brucelosis are caused by *B. abortus* and *B. melitensis.* But because of genetic similarity

Table 2. Reactivity of the sera in ELISA (−, negative serum; +, positive serum).

No. of person	*Brucella abortus*	*Coxiella burnetii*	*Leptospira* sp.	*Rickettsia conorii*	*Leishmania infantum*	*Hantavirus*	*Hepevirus*
1	-	-	+	+	-	-	-
3	-	-	-	+	-	-	-
7	-	-	-	+	-	-	-
8	-	-	+	-	-	-	-
10	-	-	+	-	-	-	-
17	+	-	+	-	-	-	-
21	+	+	-	+	-	-	-
22	+	+	+	-	-	-	-
23	+	-	-	-	-	-	+
26	+	-	+	+	-	-	-
29	-	-	+	-	-	+	-
30	-	-	+	-	-	+	-
31	-	+	-	-	-	-	-
32	-	-	-	-	-	-	+
33	-	-	+	+	-	-	-
36	-	+	-	-	-	-	-
37	-	-	+	-	-	-	-
42	+	-	+	-	-	-	-
43	-	-	-	-	-	-	+
48	-	-	-	-	-	+	-
49	-	+	-	-	-	-	-
50	-	+	-	+	-	-	-
60	-	+	-	-	-	-	-
64	-	-	+	-	-	-	-
66	-	+	-	-	-	-	-
67	-	-	+	-	-	-	-
68	+	-	+	-	-	-	-
∑ (pos.)	7	8	14	7	0	3	3
% (pos.)	10	11.4	20	10	0	4.3	4.3

The sera no. 2, 4−6, 9, 11−16, 18−20, 24, 25, 27, 28, 34, 35, 38−41, 44−47, 51−59, 61−63, 65, 69 and 70 did not react with any of the seven antigens and are not given in the Table.

within the Brucella genus, we can assume that the different species will have the same or similar major outer membrane proteins (MOMP) [9]. The presence of brucellosis in the Middle East is endemic. Afghanistan belongs to countries with the highest incidence of brucellosis [10]. Some cases of brucellosis in the military units were described [11, 12, 13].

Coxiella burnetii – out of 70 tested sera, 8 sera were positive (11 %). Q fever is quite common in the Middle East and in Mediterranean. Because of many possible routes of transmission, the probability of infection in endemic regions is very high. Špliňo et al. [14] described an epidemic among Czech soldiers in Bosnia. Unfortunately, the literature about Q fever in Afghanistan is limited. Arsen´eva [15] tested animal sera. Among troops, authors mostly described cases from Iraq [16, 17, 18].

Leptospira spp. – out of 70 tested sera, 14 sera were positive (20 %). The producer states that this ELISA kit should detect genus-specific human antibodies against *Leptospira*. But the classification of the genus Leptospira, with so many serovars, is difficult so it is not possible to identify specific serovar in the positively reacting sera. Leptospirosis is dangerous for soldiers operating in swamps [19]. Pappas et al. [20] mentioned that there are no data from countries suffering from wars. Bryan et al. [21] tested 570 sera from soldiers in Pakistan and they found antibodies in 1-6 % of them. Mansour-Ghanaer et al. [22] found 74 cases of leptospirosis in Iran.

Rickettsia conorii – in this kit, the antigen was obtained from *Rickettsia conorii*. The producer states that this antigen reacts equally with *R. ricketssii*, *R. slovaca* and *R. africae*. We found 7 positive sera (10 %). It is known that there are two rickettsial diseases in Afghanistan – Siberian tick typhus and Mediterranean fever. Many authors described just the presence of different rickettsial species but only a few authors focused on the evidence of the disease [23, 24, 25, 26, 27].

Leishmania infantum – we found no positive sera. The reason of our result is that *L. infantum* causes visceral leishmaniasis which is found only sporadically in Afghanistan [28, 29]. Visceral leishmaniasis can be found in Iran or Pakistan [13]. On the other hand, Afghanistan is known for the presence of cutaneous leishmaniasis [30, 31, 32, 33].

Hantavirus – out of 70 tested sera, 3 sera were positive (4 %). This kit uses recombinant antigen of three hantaviruses (Hantaan, Dobrava and Puumala). The cases of HFRS were known from the past (Bosnia - Stuart et al. [34]; Croatia - Mulic and Ropac [35]). The literature about the Middle East is limited and sometimes the authors differ in their statements [4, 36].

Hepevirus – out of 70 tested sera, 3 sera were positive (4 %). The most cases of hepatitis E occur in south Asia [37], where the contaminated water is a usual source of infection. But without laboratory confirmation, it is very difficult to distinguish hepatitis E from other viral hepatitides [37]. About the situation in Afghanistan or in the soldiers, there are limited data [38, 39, 40]. Carmoi et al. [41] tested Afghan population and they found high prevalence of antibodies – 28.4 %. Other authors focused on neighboring countries (Pakistan, Iran), so we can assume that hepatitis E might be endemic [42, 43, 44, 45].

For diagnosis of some zoonotic diseases, ELISA method is not available or ELISA method is not used at all (cutaneous leishmaniasis).

CONCLUSIONS

In this study, we focused on detection of specific IgG antibodies against selected zoonotic agents with which the Czech soldiers can come across during their mission in Afghanistan. We tested blood samples from 70 returning soldiers by commercially available ELISA kits. Seven sera (10 %) reacted with antigen from *Brucella abortus*, eight sera (13 %) reacted with antigen from *Coxiella burnetii*, fourteen sera (20 %) reacted with antigen from *Leptospira* spp., seven sera (10 %) reacted with antigen from Rickettsia spp., no serum reacted with antigen from *Leishmania infantum*, three sera (4 %) reacted with antigen from Hantavirus and three sera (4 %) reacted with antigen from hepatitis E virus.

After their arrival, all soldiers were medically examined and we know that all soldiers, we tested, were found healthy. We have no information about their position or health condition during their mission and about their previous missions. We can only assume that they came into contact with selected zoonotic agents during their last mission in Afghanistan.

In literature we did not find enough data about the epidemiological situation in Afghanistan (including zoonoses), probably due to the war conflict. According to our results we can assume that in Afghanistan there is a chance for possible infection of soldiers or travelers with some zoonotic agents. In many cases these organisms cause only fever or the infection could be asymptomatic and the proper laboratory diagnosis should rely on the combination of cultivation, microscopy, molecular techniques and not only on serological data.

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