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Screening of the Prevalence of Antibodies to the Tick *Hyalomma lusitanicum* in a Province of Northern Spain

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1. Introduction

In the recent decades it has been found the occurrence of a large number of hitherto unknown or undervalued pathogens, and they present a risk to health and human welfare. Almost all incidents caused by emerging pathogens have been attached to zoonotic agents, which expanded its host range and are capable of breaching the species barrier (Zeier et al., 2005). Besides the known diseases, new ones emerge or reemerge due to a variety of socioeconomic, health and environmental questions. This increase in communicable diseases has serious implications for public health and animal health (Chomel, 1998; Daszak et al., 2000, Cleaveland et al., 2001, Simpson, 2002; Daszak & Cunningham, 2003; Zeier et al., 2005; Cunningham, 2005; Blancou et al., 2005, Gibbs, 2005; Gortázar et al., 2007).

Emerging zoonoses are also a public health problem, the biggest threat to animal welfare, environmental quality and conservation of biodiversity (Daszak et al., 2000; Cunningham, 2005, Briones et al., 2002). The expected increase contact between humans and wildlife, caused by anthropogenic interference in the ecosystem, increase the emergence of pathogens originating in wildlife cycles, which can cross-infect man and animals (Bengis et al., 2004). Thus, wildlife is a constant source of "zoonotic pool" that plays a fundamental role in human exposure to infectious agents against novel animal (Morse, 1995).

In the recent past, the diseases of wild animals have been important only when they threatened livestock or human health (Daszak et al., 2000), but outbreaks in endangered species have led to them having more significant consideration. Currently, these diseases are booming, especially in the space where interaction occurs between wildlife and farm animals, including an increase in contact between them and the man (Simpson, 2002; Gortázar et al., 2007). Arthropod-borne diseases represent the most common zoonosis in relation to wildlife in the northern hemisphere, especially the Old World (Lindgren et al., 2000), so infectious agents are diverse and constantly growing, so their relationship would be endless (Bueno et al., 2009).

Wild animals and arthropod vectors play also important roles in the exposure of humans and domestic animals to animal-borne pathogens (Morse, 1995). Contact between humans and wild animals may occur when people venture into the latter's ecosystems, such contact in foreseen as a future generator of cross infestation, but the knowledge of ectoparasites, with special mention to ticks and their hosts' reservoirs that are located in many areas of

world are unstudied. Ticks are vectors of disease have a wide range of pathogens (bacteria, rickettsia, viruses, protozoa and nematodes), affecting both domestic and wild animals, and humans with a zoonotic character. Ticks are considered as one of the most efficient arthropod vector role (Hillyard, 1996, Wall & Shearer, 1997).

In fact, it's known that ticks are vectors of transmission of a number of human viruses that causes Tick-borne meningoencephalitis, Colorado tick fever and Crimean-Congo hemorrhagic fever, among others, bacteria (*Rickettsia* spp, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Francisella turalensis*, among others) (Lopez-Vélez & Molina, 2005; Toledo et al., 2009) and protozoan pathogens including parasites (Lledó et al., 2010) and control of ticks and tick-borne diseases is a major component of animal health programes for the protection of livestock. Over the ixodid tick species which are often found on humans exposed to infested vegetation *Amblyomma* species, *Dermacentor* spp, *Haemaphysalis* spp, *Ixodes* spp and *Hyalomma* spp are found (Estrada- Peña et al., 1999).

In this sense, Genus *Hyalomma* is a phylogenetically young group of ixodid ticks. As proposed Kolonin (2009), domestication and the development of cattle-breeding stimulated the evolution and biological progress of this group. These transformations continue to this day, as is apparent from the great number of intraspecific forms. *Hyalomma* ticks (Figure 1) are medium to large sized, with prominent mouthparts. Most species are 3 hosts, but there are also 1 and 2 hosts. Some species of this genus can use 1, 2 or 3 hosts to develop according to the host they found. The life cycle can last between 3-4 months and more than a year, depending on species and climate. The nymphs and adults stay overwinter in cracks and crevices between the stones of walls and barns, or uncultivated grasslands. Adults are found throughout the year, although the parasite load is higher in spring and summer, parasitizing deer and wild boar. Larvae and nymphs parasitize rabbits and are more prevalent in spring. Though this genus is usually restricted to the Mediterranean region, one of the species, *Hyalomma lusitanicum,* Koch 1844 (*Ixodoidea: Ixodida*) has a widespread distribution in some regions of Southern Spain, from which is introduced by wild animals (Encinas-Grandes, 1986).

Fig. 1. Adult of Hyalomma lusitanicum

This ixodid tick is also located in the Burgos province (north western Spain) (Cordero del Campillo et al., 1994), in areas mainly rural, though recreational activities attracting non-residents have increased in recent years (Figure 2). This tick is not the most prevalent tick in this area.

In this studied area, in the northern sector, the winters are cold and humid and the summers are cold. Vegetation under Atlantic influences consists in oakwood and beechwood, with brushwood. In the southern sector is submediterranean and shows similar winter but hottest summers. Vegetation consists in gall-oak groes and holm-oak wood, being brush scarce (Roman et al., 1996; Dominguez, 2004). Mean summer temperatures in this area range between 16 and 20ºC, while mean winter temperatures range between 2 and 5ºC. Rainfall is usually high in winter at some 900-1100 mm/year. Altitude is ranging between 600-800 meters on the plateau. Climatic differences among different areas of Spain are responsible of both, the diversity of tick species and the circulation of tick-borne pathogens.

Fig. 2. Landscape of the studied area

While ticks have a clear geographical distribution in relation to climate, temperature, humidity and like attitude, climate change and global warming have influenced the geographical distribution of ticks. So, some groups as Experts from the International Scientific Working Group (ISW-TBE) on Central European encephalitis transmitted by ticks (Tick-Borne Encephalitis) warn of the first detection of these arthropods in areas above 1,500 m above sea level.

In Spain, it looks to be *Ixodes ricinus* the most abundant and widespread tick in the Basque Country (Northern Spain) (Barandika et al., 2006) and some authors (Toledo et al., 2009) have observed that one of the most abundant species in Central regions of Spain in terms of infection and tick abundance is *Hyalomma lusitanicum*. However, as happens with other ectoparasites and their host-reservoirs, are located in many areas of Spain that are unstudied or missstudied.

In this sense, the genus *Hyalomma* is one of the vectors for *Theileria annulata* that causes Mediterranean theileriosis, and produces considerable economic losses in cattle (Viseras & Garcia Fernandez., 1999) though in terms of public health, this tick is considered as not anthropophilic.

Tick bites are generally painless and many people may not even notice the bite and may never find the tick if it falls off. The majority of individuals with tick bites develop no symptoms, and many do not remember getting bitten. The direct damage caused by ticks depends on the number, species and location of the parasites. However, the most harmful effects on animal and public health are derived from indirect vector character (Hillyard, 1996, Wall & Shearer, 1997; Encinas et al., 1999, Sonenshine et al., 2002). Damage can be

divided, according to the scope of the consequences, cutaneous and systemic. We can point to the inflammatory reaction in the fixation point, which causes itching, scratching, excoriations and self-harm. The reaction can spread awareness of the antigenic components of saliva, causing even anaphylactic shock. The bites often become infected with pyogenic agents such as *Staphylococcus aureus*, not ruling out the occurrence of myiasis (Wall & Shearer, 1997; Encinas et al., 1999, Wall, 2007).Systemic effects include tick paralysis is caused by a neurotoxin secreted by the females of some species, producing a neuromuscular blockade (Sonenshine et al., 2002). *Ixodes ricinus* and *Haemaphysalis punctata* are two species in our area involved in the process of paralysis (Hillyard, 1996, Wall & Shearer, 1997; Encinas et al., 1999). The mechanical transmission of pathogens from sepsis occurs in tick infestation in lambs or calves (Kettle, 1995), not neglecting the effects of blood loss. In this sense, a female has been eating packed up to 4 g of blood, so intense in parasites are common anemias (Encinas et al., 1999).

When ticks bite at time of the attachment they inoculate saliva and occasionally, a neurotoxin secreted at the time of attachment. Saliva in feeding ticks is rich in several biochemical components including various enzymes (Sauer et al., 1995; Giménez-Pardo & Martínez-Grueiro, 2008). Immunogenic and pathogenic proteins enter in the mammalian host during feeding via the tick salivary gland (Kaufman 1989). Their saliva secretions during bites are capable to produce toxicoses and allergic reactions, and in animals it's known that ticks are capable to induce a high humoral immune response (Perez-Sanchez et al., 1992). In human tick attachment are brief and sometimes, are immature forms which introduce a low quantity of antigens which are not enough to induce a good humoral response.

In this paper we i) study the preliminary humoral response in humans, employing antigens from male and female *Hyalomma lusitanicum* tick as a previous work to know if this tick could in a future be capable to bite humans in rural regions being implicated in the transmission of human pathogens and ii) observe differences between males and females in the response that could induce when they bite humans. For this, it was assayed a panel of human sera of different days post bitten by indirect enzyme-linked immunoabsorbent assay technique. This study was carried out in Burgos province (Spain) and results are compared with those obtained from general population and without history of tick bites**.**

2. Material and methods

2.1 Parasites

465 unfed adult ticks (males and females) were collected from vegetation in spring and each one was identified by binocular lens. Males (190) were separated from females (275) and processed individually. Specimens were immersed in 70% alcohol for ten minutes rinsed for 30 seconds in Milli-Q water and dried in a filter paper. Each ixodid tick was transferred in PBS-saline (10mM PBS pH 7.2, 146mM NaCl) in a Potter-Elvejem on an ice bath and homogenized. Extracts were removed in eppendorf tubes and centrifuged for 1min at 500 rpm to deposit cuticle fragments and tissue rests. Supernatants were collected and centrifuged in new eppendorf at 14000 rpm for 5 min, filtered through a 0.22µm filter (Millipore) and the resultant one were again filtered through a 0.22µm filter. This whole tick extract was collected and the protein concentration determined using the technique of Bradford (Bradford, 1976) and subsequently adjusted to 1mg/ml for females and 0.635 mg/ml for males using PBS-saline.

2.2 Human sera

The study was carried out on two population groups: sera from people who had been bitten by ticks and serum samples from the general population with no history of tick bites as control group. For the first group, 42 samples of human serum from 23 patients bitten by ticks were randomly collected from patients to the Burgos Health Centres (Figure 2), and the following information was recorded for each person: age, sex, occupation and area of residence. A serum sample was withdrawn from all bitten patients at the time of consultation and the patients were asked to return to provide another sample at 15-20 days later. All residents living in the study area are people related to livestock, or have pets in their care. They are people with an epidemiological history of tick bite, but without identification of the same. For the second group, serum from 97 people was obtained from the general population presented to healthcare centres for reasons unrelated to infectious diseases. All samples were aliquoted and conserved at -20ºC until use. The survey was performed with the consent of the subjects included in the study, and in compliance with the ethical standards of Alcalá de Henares University´s Comitte on Human experimentation, as well as with the Helsinki Declaration of 1975 as revised in 2004.

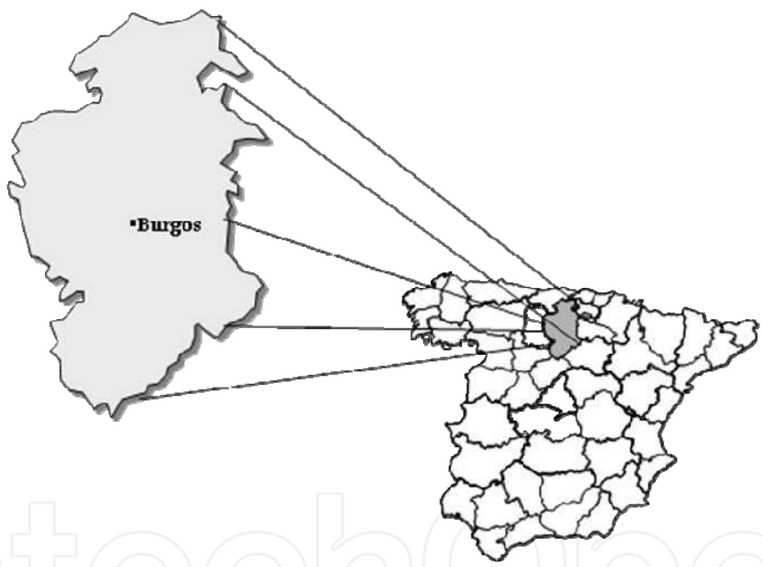

Fig. 3. Location in the Spain map the Burgos province

2.3 ELISA reactions

An indirect ELISA technique was used to detect anti-ticks Abs (IgG, IgM, IgE) in the samples. As antigens were used protein extracts from both males and females of *H. lusitanicum*. ELISA plates were coated with 1µg of antigen per well diluted in 0.1M carbonate/bicarbonate buffer, pH 9.6 and incubated at 4ºC overnight. It was realized three washes in 0.05% PBS-Tween 20. After incubation with 0.05% PBS-Tween 20-1% casein for 1h at 37º and the subsequent washes, 100µl per well of human sera diluted at 1/50 for IgG, 1/20 for IgM and 1/10 for IgE in 0.05% PBS-Tween-20 and incubated for 1h at 37ºC was added. After the subsequent washes, peroxidase-labeled anti-human immunoglobulin antibodies were also diluted in 0.05% PBS-Tween at 1/4000 for both anti-IgG (CalBiochem),

anti-IgM (CalBiochem) and 1/500 for anti-IgE (CalBiochem), incubated for 1h at 37ºC. ABTS (2-2´-Azino bis(3-Ethylbenzhia-zoline-6-sulfonic acid) and H_2O_2 were used as substrates. Reactions were stopped after with 3N sulphuric acid and results were read on a spectrophotometer at 405nm. On each ELISA plate were included negative controls sera obtained from people that never have been bitten by any tick. Experiences were done in triplicate (or more depending on the sera volume). The sample sera were considered positive when their optical density surpased a treshold calculated as the mean optical density of the negative control sera plus three times the standard deviation (mean OD+3δ). The same parameters were employed when it was referred to the general population.

3. Results

Twenty three of the patients presented at the Burgos Health Services for tick bites were asymptomatic at the time of sampling. Of these patients, 12 were males and 11 were females (Table 1).

	Age-groups (years)		
	0-10	**11-59**	**60-97**
Males (%)	2 (8.69)	4 (17.39)	6 (26.08)
Females (%)	2 (8.69)	8 (34.78)	1 (4.34)

Table 1. Characteristics of the patients attended for tick bites in this study

Nine patients were bled once, 13 patients were bled two times and 1 patient was bled in three occasions. All patients were bitten by unknown tick species and in 9 of them the serum samples were withdrawn more than 21 days after the tick bite (Table 2). There was no relation between the tick bite and the age, profession or place where the patient lived.

Of the patients studied, 4 had Abs (IgG) against both *H. lusitanicum* male and female antigens and two of them reacted only with male but not with female whole tick antigen as indicated in Table 2. Two sera showed Abs (IgG) against female but not male *H. lusitanicum* antigens.

Because ticks could have been removed from people in the first 48h post-attachment and even in the case of an effective transmission, the patients could have been bled before the synthesis of the anti-tick Abs have occurred, low values of IgM were found. Afterall, sera from three patients were considered positive against female antigens.

Serum from one patient was positive against male antigens of *Hyalomma lusitanicum.* Two patients showed IgM positive as only response and this fact indicate a possible acute case of *H. lusitanicum* bitten (see in Table 2).

Antibodies to IgE were analyzed in those sera that were positive against both male and female antigens, but we have not observed response to anti-IgE, neither male nor female *H. lusitanicum* antigens.

Relative to the general unexposed population, none of the sera studied were positive against female antigens of *H. lusitanicum* though when sera were assayed against male antigens, one serum was positive (see in Table 3).

Patient (nº)	Sera (nº)	Days (p.b)[1]	IgG (X±SE)		IgM (X±SE)	
			Female (Ag)	Males(Ag)	Females(Ag)	Males(Ag)
1	**5**		1.235±0.046	**2.194±0.144**	0.140±0.014	0.112±0.031
2	**6**		0.856±0.021	0.785±0.074	0.086±0.002	0.163±0.043
	7		0.975±0.077	1.098±0.207	0.079±0.022	0.165±0.022
3	**8**		1.270±0.094	1.224± 0.161	0.059±0.009	0.148±0.002
4	**9**		**1.760±0.058**	**1.903±0.165**	0.102±0.017	0.122±0.028
5	**17a**	17	1.044±0.015	0.928±0.133	0.106±0.009	0.144±0.038
	17b		0.816±0.040	0.499±0.062	0.100±0.026	0.057±0.027
6	**23a**		0.946±0.113	1.199±0.132	**0.399±0.025**	0.023±0.002
	23b	25	1.291±0.201	1.268±0.111	**0.350±0.033**	0.144±0.046
7	**36a**		0.724±0.067	0.445±0.095	0.060±0.001	0.050±0.007
	36b	11	1.442±0.124	1.229±0.024	0.046±0.010	0.134±0.019
	50	20	1.033±0.061	0.516±0.036	0.050±0.012	0.121±0.021
8	**80**		1.450±0.050	1.359±0.072	0.068±0.012	0.166±0.015
	79		1.443±0.190	1.250±0.138	0.107±0.004	0.187±0.005
9	**81**		1.161±0.093	0.674±0.066	0.023±0.004	0.150±0.016
	82		0.404±0.042	0	0.062±0.004	0.175±0.031
10	**84**		0.926±0.112	0.707±0.166	0.041±0.010	0.173±0.042
	83		1.241±0.011	0	0.040±0.004	0.171±0.039
11	**189**		1.256±0.187	1.040±0.140	0.100±0.011	0.166±0.041
	190	22	1.378±0.184	1.136±0.031	0.099±0.004	0.166±0.040
12	**270**		0.743±0.042	0.714±0.120	0.137±0.002	0.226±0.039
	271	33	0.872±0.009	0.626±0.176	0.146±0.016	0.238±0.025
13	**272**		1.289±0.161	1.164±0.038	0.068±0.004	0.060±0.007
	273	25	1.057±0.074	0.766±0.072	0.077±0.010	0.097±0.009
14	**279**		**1.991±0.018**	**2.139±0.031**	0.150±0.010	0.228±0.017
	280	30	**2.152±0.029**	**1.767±0.029**	0.119±0.009	0.290±0.032
15	**258**		**1.730±0.047**	**1.876±0.205**	0.091±0.009	0.256±0.032
	259	13	**1.750±0.051**	**1.496±0.150**	0.107±0.007	0.259±0.045
16	**290**		**1.625±0.095**	**1.936±0.088**	0.136±0.011	**0.371±0.025**
	291	27	**1.628±0.070**	**1.864±0.101**	0.114±0.007	**0.316±0.020**
17	**293**		1.283±0.033	0.931±0.078	0.029±0.0007	0.055±0.002
18	**303**		0.854±0.073	0.324±0.047	0.089±0.014	0.159±0.009
	304	18	0.883±0.074	0.517±0.046	0.055±0.007	0.156±0.018
19	**308**		1.531±0.070	1.104±0.070	0.030±0.012	0.134±0.006
	309	20	1.309±0.098	0.833±0.045	0.054±0.008	0.110±0.025
20	**313**		0.974±0.019	0.310±0.026	**0.149±0.010**	0.109±0.015
21	**314**		1.589±0.241	0.512±0.044	0.101±0.014	0.077±0.010
	315	24	**1.891±0.055**	0.493±0.007	0.120±0.010	0.116±0.026
22	**388**		0.624±0.054	0.309±0.037	0.100±0.005	0.111±0.009
	389	22	0.664±0.070	0.088±0.026	0.063±0.007	0.127±0.021
23	**354**		1.072±0.239	1.096±0.026	0.121±0.014	0.195±0.014
	355	25	**1.711±0.054**	0.251±0.077	**0.237±0.028**	0.214±0.009
Control sera (n=8)			0.996±0.149	0.782±0.098	0.086±0.013	0.149±0.021

Table 2. Reactivity of the human sera from the patients bitten by unspecific ticks, against the *Hyalomma lusitanicum* (male or female) whole antigens.

Sera (nº) (n=3)	IgG (X±SE)		Sera (nº) (n=3)	IgG (X±SE)	
	Females	Males		Females	Males
1	1.036±0.096	0.608±0.015	**49**	0.650±0.047	0.966±0.058
2	0.897±0.149	0.895±0.048	**50**	0.585±0.072	0.967±0.036
3	0.465±0.014	1.140±0.053	**51**	0.276±0.013	**1.75±0.025**
4	0.377±0.016	0.896±0.015	**52**	0.296±0.030	0.763±0.031
5	0.887±0.096	0.643±0.100	**53**	0.479±0.046	0.570±0.053
6	1.031±0.077	0.802±0.055	**54**	0.346±0.014	0.700±0.176
7	0.279±0.043	0.593±0.088	**55**	0.299±0.005	0.676±0.022
8	0.915±0.055	0.898±0.038	**56**	0.817±0.015	0.605±0.127
9	0.630±0.043	0.425±0.045	**58**	0.343±0.021	1.225±0.067
10	0.544±0.024	0.650±0.088	**59**	0.415±0.098	0.525±0.037
11	0.378±0.056	0.802±0.065	**60**	0.349±0.042	0.519±0.123
12	0.311±0.008	0.606±0.060	**61**	0.434±0.010	0.492±0.035
13	0.447±0.061	0.554±0.025	**62**	0.430±0.012	1.023±0.008
14	0.117±0.024	0.176±0.009	**63**	0.325±0.041	0.585±0.103
15	0.386±0.017	0.884±0.060	**64**	1.040±0.024	1.156±0.193
16	0.778±0.093	0.527±0.103	**65**	0.824±0.140	0.685±0.015
17	1.019±0.114	0.613±0.038	**66**	0.424±0.012	0.722±0.094
18	0.938±0.045	0.666±0.030	**67**	0.498±0.066	1.476±0.055
19	0.234±0.007	0.314±0.017	**68**	1.077±0.081	1.004±0.082
20	0.485±0.089	0.614±0.055	**69**	0.927±0.068	0.800±0.172
22	0.671±0.048	0.466±0.045	**70**	0.234±0.042	0.461±0.107
24	0.473±0.055	0.211±0.026	**71**	0.323±0.014	0.891±0.200
26	0.383±0.055	0.250±0.058	**72**	0.876±0.030	0.446±0.130
28	0.389±0.041	0.555±0.082	**73**	0.524±0.060	ND
29	0.475±0.009	0.167±0.032	**74**	0.897±0.100	1.182±0.304
30	0.676±0.051	0.306±0.067	**76**	0.786±0.016	0.516±0.045
31	0.437±0.005	0.810±0.025	**77**	0.888±0.074	0.922±0.036
32	0.892±0.038	0.206±0.060	**79**	0.345±0.027	0.856±0.035
33	0.943±0.017	0.148±0.053	**80**	0.904±0.017	1.408±0.144
34	1.003±0.109	0.957±0.164	**81**	0.670±0.017	0.826±0.076
35	0.160±0.057	0.499±0.046	**82**	0.340±0.062	0.866±0.085
36	0.382±0.016	0.943±0.076	**83**	0.398±0.042	0.985±0.074
37	0.989±0.045	1.093±0.077	**84**	0.786±0.016	0.578±0.032
38	0.728±0.025	1.400±0.080	**85**	0.425±0.010	ND
39	0.224±0.055	1.306±0.080	**86**	0.212±0.007	0.777±0.060
40	0.752±0.067	0.970±0.136	**87**	0.322±0.061	0.671±0.016
41	0.699±0.018	0.506±0.052	**89**	1.013±0.117	0.926±0.103
42	0.556±0.019	1.043±0.228	**90**	0.408±0.091	0.950±0.176
43	0.238±0.013	0.928±0.155	**91**	0.649±0.062	0.946±0.066
44	0.389±0.029	1.012±0.147	**92**	0.891±0.016	0.966±0.117
45	0.894±0.084	1.184±0.065	**93**	0.574±0.084	1.054±0.056
46	0.785±0.089	1.320±0.077	**94**	0.218±0.057	0.658±0.035
47	0.476±0.011	1.382±0.120	**95**	0.609±0.055	1.053±0.127
48	0.528±0.033	0.857±0.106	**96**	0.438±0.055	1.462±0.250
Control sera ♀ (n=16)	0.570±0.045		**Control sera ♂ (n=18)**		0.780±0.194

Table 3. Results of the general population antibodies (IgG) against *Hyalomma lusitanicum* male and female antigens

4. Conclusion

Burgos is a province of the inner north western region of the Iberian Peninsula. Its climate is continental with cold dry winters and mild summers. Animal husbandry is a very important economical source and livestock parasitism of ticks is common in the region. *Hyalomma* spp. ticks are distributed in Africa, the Mediterranean climatic zone of southern Europe, and in Asia.

It is known that ticks are important pets and livestock transmitting tick-borrne diseases. In this sense, though humans are not the preferred hosts of *Hyalomma* ticks and are infrequently bitten in comparison to livestock, sporadic infection of people is usually caused by *Hyalomma* ticks.

In fact, Crimea-Congo Haemorrhagic Fever transmitted by *Hyalomma* spp occurs sporadically throughout many areas of Africa, Asia and Europe, but can cause mortality (Estrada-Peña & Jongejan., 1999). Recently a new group of spotted fever has been isolated from *Hyalomma marginatum marginatum* ticks in Morocco (Beati et al., 1997). In Europe have been detected genotipically similar organisms in Portugal (Beati et al., 1995), Croatia (Punda-Polic et al., 2002), Corsica (Matsumoto et al., 2004), Germany (Rumer et al., 2011) and in Spain (Fernández-Soto et al., 2003).

Hyalomma lusitanicum is called perinneal specie which is present on cattle year around. These parasitize domestic and wild animals and birds, and are usually abundant in semi-arid zones. Its distribution reflects peaks in May-June and October that corresponds to the periods of maximum activity of adults (Habela et al., 1999), but in winter specimens (males and females) remain fixed on their host without feeding (Yousfi-Monod & Aeschliman., 1986). Adult *Hyalomma* actively run out from their resting sites when a host approaches. Cattle, rabbits, hares and deer which are the hosts of *Hyalomma lusitanicum* are well represented in the studied region.

The frequency with which different tick species bite humans varies significantly from one zone to another and much it depends on the likelihood of humans entering their biotope (human contact with ticks for professional and recreational activities) and the tick affinity for humans. Climatic changes could be probable implicated in the northern establishment of ticks, but perhaps would be more dependent on the introduction of adult females on wild and domestic ruminants due to the uncontrolled movement of livestock as have been proposed recently Rumer et al., (2011) in which the only documented *Hyalomma* spp. tick in Germany was found on a human in the southern part of the country (Lake Constance area) in May 2006, but they did not ruled out the tick transportation from Spain.

However it is not easy to detect that a person has been bitten by a tick, because people may confuse the bite ticks or other arthropods that might be no elicit antibody response.

Ticks can inoculate a variety of active molecules during feeding that can block pain, reduce inflammation and suppress or modulate innate and specific acquired immune defences (Brossard & Wikel., 2004). But the duration of the tick attachment may be insufficient to allow for adequate amount of saliva to elicit a detectable antibody response. Sometimes happens that is necessary several tick exposures before the antibody response will be strongly enough to be detected by ELISA. Ticks deposit saliva at the site of their attachment

to a host in order to inhibit haemostasis, inflammation and innate and adaptive immune responses but ticks are able to modulate their host's local haemostatic reactions (Carvalho et al., 2010)

As happens with other ticks in which salivary gland proteins are immunogenic (Sanders et al., 1996), our results have shown that *H. lusitanicum* has proteins (antigens) that stimulate the production of immunoglobulins in humans as well as the finding of a significant high prevalence in bitten patients by ticks respect to the control group (see in Tables 1 and 2). It would be very interesting to conduct an epidemiological study, in those sera that have positive results, if at any time have had a history of febrile illness of unknown etiology with or without rash.

As occurs with other genus or species of ticks capable to induce high responses, *H. lusitanicum* could share antigens with other ticks. It is easy to found that antigens can cross react with the antigens of a closely related species. This fact has been observed in several studies that have realized cross-resistance studies between *Dermacentor andersoni* and *Dermacentor variabilis* (McTier et al., 1981), *Hyalomma anatolicum anatolicum* and *Boophilus microplus* (Parmar et al., 1996), *H. a. anatolicum, Hyalomma dromedari* and *Boophilus microplus* (Kumar & Kondal., 1999) as well as between a series of other tick species (Brown & Askenase., 1984; Jaansen van Vuuren et al., 1992). In fact, the homology among several tick species suggests that possibly common antigen(s) may be suitable for a vaccine against some different ticks.

By other hand, though ticks are well known blood suckers, blood sources between males and females seem to be different and gene expressions in fedding males and females are also different (Aljamali et al., 2009). In fact the male blood meals may be digested and nutrients can be used for spermatogenesis. The host blood meal is necessary for egg production in female ticks (Sanders et al., 1996). As occurs with partially fed female *Ixodes ricinus* (Linnaeus, 1758), female and male *Amblyomma variegatum* (Fabricius, 1794) and *Rhipicephalus appendiculatus* Neumann, 1901 in which have been observed that exist species- and sex-specific differences in the effects of tick salivary gland antigens on human lymphocyte proliferation, our results, as can be seen in Table III, have demonstrated that exist differences in the human antibodies response against male and female *H. lusitanicum* antigens. This fact makes that both could be considered as susceptible to bite humans.

Since for each tick-borne disease there may be one or several vectors (Lane, 1994), perhaps this ixodid could be implicated as a vector susceptible of parasitizing humans. In this sense, other studies would in a future provide us about the frequency and which life cycle stages of this tick can infest humans as well as its role in the transmission of human pathogens as happened with those studies in which *Dermacentor marginatus* was recently demonstrated to be the vector in the transmission of *Rickettsia slovaca*, that causes the TIBOLA/DEBONEL disease in humans (Rehacek, 1984; Lakos., 1997; Raoult et al., 1997; Ibarra et al., 2006).

Until date, each tick species has preferred environmental conditions and biotopes that determine the geographical distribution of the ticks and the risk areas fro tick borne diseases, but day to day more research studies are going on in order to elucidate a higher diversity of ixodid tick species infesting humans potentially transmisors of underdiagnosed diseases.

In this sense, we consider it would be very interesting to educate primary care physicians in these areas, to be able to identify ticks and how clinicians should deal with patients who have been bitten by ticks, because it would be very interesting to discover potential transmitters of both old and new diseases. It is necessary to develop studies in which ticks removed from the patients must be directly preserved in ethanol and identified because the knowledge about tick species that are susceptible of parasitizing humans is essential for assessing the risks for people who become infected, we can detect pathogens and design measures to prevent infection.

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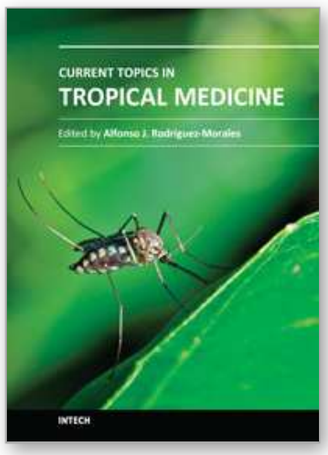

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