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USE OF SCUTAL INDEX OF THE TICK HYALOMMA LUSITANICUM (ACARI, IXODIDAE) TO INFER FEEDING DURATION

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Abstract An increment in human tick bite cases has been noticed in Eastern Spain in the last years. Three tick species has been detached from patients assisted at Vall d'Alba Heath Center during 2018 and 2019: *Dermacentor marginatus, Hyalomma lusitanicum* and *Rhipicephalus sanguineus*. *H. lusitanicum*, with 204 tick bite cases (86.8%), has been the preponderant tick on humans. Of them, 116 females has been used to analyze the scutal index (SI) like the relation between the idiosoma length and the scutum width. The SI of *H. lusitanicum* ranges from 1.67 to 9.09, being the 80.2% of females belonging to the group of low SI value (1.67-2.22) and 6.0% of females that reaches the maximum grade of engorgement (SI 3.38-9.08). The scutal index, because of the tick engorgement during the feed period, may be used to estimate the time that ticks remain attached to human patients thus assisting with the assessment of pathogen transmission risk.

Key words vector, feed duration, engorgement, pathogen infection risk.

INTRODUCTION

Ticks (Acari: Ixodida) are blood-sucking arthropods living as ectoparasites on several groups of vertebrates, but they incidentally also turn to humans as a host. Due to this feeding behavior, these ixodids are very efficient carriers of many types of pathogenic agents such as protozoans, bacteria and viruses. Ticks are currently considered to be, second only to mosquitoes, the most important group as vectors of human infectious diseases in the world (de la Fuente et al., 2008). The species *Hyalomma lusitanicum* Koch, 1844 is distributed through the western Mediterranean Basin. The flat adult ranges between 5-6mm in length when unfed. Its one year cycle includes three hosts from two species. Larvae and nymphs feed on lagomorphs but adults usually feed on domestic and wild ungulates particularly cervids and bovines. All stages are more active along spring and summer, and adults show a peak in April-July. The host seeking behaviour is based in an energetic hunter strategy. This tick has been reported from humans (Estrada-Peña et al., 2017).

H. lusitanicum is involved in the transmission of a number of pathogens. The vectorial capacity has been proofed to protozoans (*Theileria* spp.), apicomplexans (*Babesia* spp.) and bacteria like Rickettsiales (*Anaplasma* spp., *Ehrlichia* spp.), Legionellales (*Coxiella burnetii* - agent of Q fever), Spirochaetales (*Borrelia burgdorferi* - agent of Lyme disease) and Thiotrichales (*Francisella tularensis* - agent of Tularemia) (Estrada-Peña et al., 2017). This tick is also vector of the Crimean-Congo haemorrhagic fever virus. In fact, *H. lusitanicum* ticks has been detected in Spain as vectors of this virus in relation with red deers (Estrada-Peña et al., 2012; Negredo et al., 2019) and where the virus has been the cause of fatal cases in humans although the identification of the vector was not corroborated being very probably hyalomma ticks (Negredo et al., 2017).

The risk of developing any tick-borne disease in humans after a tick bite depends of several factors, including among others the duration of tick feeding period. Several studies have estimated a significant positive correlation between attachment duration of tick and disease symptoms. Long attachment period after 48-72 hours facilitates the pathogen transmission to the host and then the potential symptomatic infection, which has been demonstrated both in animal or human studies (Piesman, 1993; Sood et al., 1997; Tijsse-Klasen et al., 2011).

The duration of tick attachment can be determined based on the named scutal index which is a measurement of the engorgement degree when females and nymphs feed on the host because of the exponential increase in body size with the ingestion of blood and the inflexible scutum state (Falco et al., 1996). The scutal index is estimated as the relation between the idiosoma length (from the anterior edge of the scutum to the posterior tip of the opisthosoma) and the scutal width (maximum width of the scutum). The scutal index value for each tick is useful to calculate the duration of tick attachment in time groups of hours (Sood et al., 1997; Gray et al., 2005; Meiners et al., 2006).

An unusual increase in the number of the people treated because of tick bites in Health Care Centers of northern of Valencian Autonomous Region (Province of Castellon - Spain) detected over the years 2018-2019 has led to further investigations of the incidence of ticks on humans.

The objectives of the present study are to explore if there is a species of preponderant tick that attacks people in the geographic area considered, and if the population of this or these ticks shows a differential range of scutal index which indicates several attachment periods on the human patients.

MATERIALS AND METHODS

Throughout 2018 and 2019 a number of ticks was collected on patients with one or more ticks attached to their body who have requested medical consultation at Vall d'Alba Health Center (central region of the province of Castellon - Eastern Spain). This medical center serves several nearby towns with a total of 12569 inhabitants. Ticks were carefully removed by grasping the capitulum with soft forceps and pulling gently. They were immersed in 70% ethanol and sent to the University of Valencia. These specimens are valid for measuring the scutal index because alcohol does not retract the body of these hard ticks (Tijsse-Klasen et al., 2011; Estrada-Peña, personal communication).

Individual scutal index was measured with an ocular micrometer fitted to a stereomicroscope (Leica/Wild M3Z Stereo Microscope). The scutal index used is the ratio between the length of the female tick idiosoma and width of scutum. The first increases during feeding whereas the scutum remains constant. Processing and representation of the data were worked with the spreadsheet Microsoft Excel for Windows (Excel 2016 (v16.0)).

Ticks were determined to species level using the identification guide by Estrada-Peña (2017). All specimens were deposited at the Colección Entomológica de la Universidad de Valencia.

RESULTS

The identification of the ixodid ticks has resulted in three species: *Dermacentor marginatus* (Sulzer, 1776), *Rhipicephalus sanguineus* (Latreille, 1806) and *Hyalomma lusitanicum* Koch, 1844. These species have been responsible of a total of 235 cases of ticks bites on humans who were treated at the Health Center of Vall d'Alba during the years 2018 and 2019. Of the total number of cases, *D. marginatus* have caused 4.3% (n= 10) of tick bites, *R. sanguineus* reached 8.9% (n= 21) and *H. lusitanicum* was clearly the preponderant tick with 86.8% (n= 204) of the attended cases. All specimens of *H. lusitanicum* were adult ticks, of them 40.7% (n= 83) were males, which do not feed nor engorged on the host, and 59.3% (n= 121) were females that engorged the body as they feed attached to the patient. The numbers of the two other species were too scarce to continue its analysis.

A total of 116 females of collected *H. lusitanicum* have been valid to determine the scutal index, or relation between the idiosoma length (LI) and the scutum width (WS) (Figure 1).



Figure 1. Chart of grouped columns: Scutal Index (SI) (Length of Idiosoma / Width of Scutum) *Hyalomma lusitanicum* females collected on human patients at Vall d'Alba Health Center during 2018-2019 . Y axis = values of SI; X axis = individual SI for each tick (n = 116). A group of 93 ticks have showed a scutal index ranging from 1.67 to 2.22 in a continuous little variable distribution. A slight jump occurred for other 7 ticks with barely increase between 2.44 and 2.50. Then the scutal index began to increase sharply from 2.56 to 3.33 in 9 ticks and more significantly from 3.83 to 5.61 in other 5 ticks. The maximum engorgement was reached by 2 ticks with a scutal index of 8.92-9.08.

DISCUSSION

Hyalomma lusitanicum has resulted the main species tick on humans in the geographical area of province of Castellon in Eastern Spain, unlike what happens in Northern Europe where the most common tick vector of human and animal diseases is *Ixodes ricinus* (Linnaeus, 1758) (Gray et al., 2005), but similarly to the widespread presence of *H. lusitanicum* in Central Spain but attacking wild animals there (Negredo et al., 2019).

Knowledge of the duration of tick feeding can be estimated from changes in body dimensions during engorgement and may contribute to assessment of disease transmission risk. Ixodid ticks usually suck in the blood intake 24 hours after the attachment and then they can potentially transmit pathogens during the prolonged feeding. So, there is an increment of pathogen infecting risk after 24-48 hours attachment.

Usually the risk following to tick bite has been determined for *I. ricinus* (Meiners et al., 2006; Tijsse-Klasen et al., 2011) but as far as bibliography data allow the determination of the engorgement level calculating the scutal index (SI) is a novel procedure in case of *H. lusitanicum*.

In present study, four tick groups could be pointed in relation to its SI: 80.2% of ticks show a SI up to 2.22, second 6.1% has a SI up to 2.50, third 7,7% has a SI up to 3.3, and fourth 6,0% is a group of ticks with maximum engorgement and SI up to 9.08. Each one of these groups could be associated to the model presented by Gray et al. (2005) of 24, 48, 72 and more than 72 hours of feeding time.

Scutal index is easy to measure, simple and reliable parameter to calculate tick feeding duration. It is applicable to *Hyalomma lusitanicum*, a tick of medium-large size that has been proven attacking humans in Eastern Spain in the last three years. Determination of the scutal index leads to identify high-risk tick bites and help to decide clinical procedures of human patients in medical centers.

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