European Bison Conservation Newsletter Vol 3 (2010) pp: 75–84

Analysis of the reasons for differences in topical specificity among various species of tick (Acari, Ixodidae) infesting European bison

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Abstract: Ixodidae are usually oligoxenic or polyxenic, hematophagous, and periodic parasitic arthropods that occur commonly throughout populations of various vertebrate species, including European bison. Little remains known about how host and topical specificity develop. Ticks are usually showing clear preferences in the choice of location at hosts. On large ungulates ticks are usually noted in the inguinal regions, laterally on the body, and neck. However, in bison, the marsh tick *Dermacentor reticulatus* is usually located at the tops of the pinnas on bison. The areas of bison skin included in the current study were chosen because either ticks were noted in them or there were distinct changes on the skin that indicated that ticks had fed there previously. The changes resulting from the feeding of various tick species were noted and the bison skin thickness in correlation with the sizes of the ticks' mouth parts was analyzed. They stated, that gnathosoma adulti of *D. reticulatus* it is 1.6 – 1.7 times shorter, than of female *I. ricinus*. Ticks do, however, exhibit distinct topical specificity during feeding. While the reasons for topical specificity vary, it does appear that in the case of *D. reticulatus* a significant factor is the correlation between the length of the gnathosoma and the skin thickness of the host, which determines the availability of the circulatory vessels.

Key words: ticks, European bison, deer, parasitism

Introduction

Ticks (Acari, Ixodida, Ixodidae) are parasitic arthropods that occur commonly throughout populations of various vertebrate species, including ungulates. These parasites are usually oligoxenic or polyxenic, hematophagous, and periodic occurring on hosts while feeding. To date, investigations have focused on tick strategies for finding hosts and feeding as well as a range of other adaptive mechanisms that permit them to infest vertebrate hosts with complicated physiology and complex hemostatic systems. The evolution of hematophagous ticks, which refers to the mechanisms that shape the creation and range of host specificity, has also been investigated (Mans, Neitz 2004). Little remains known, however, about how topical specificity develops.

Distinct parasitic topical specificity is noted among ungulates, including the European bison (e.g., Kadulski 1989; Izdebska 2004). In Poland nineteen species of tick are considered to be permanent elements of the fauna (Siuda

2008) including the typical bison parasite *Ixodes ricinus* (Linnaeus, 1758), known commonly as the sheep tick, the castor bean tick, or the European castor bean tick. This is the most commonly observed Ixodidae tick species, which attacks the majority of Polish land vertebrates. It is noted on bison relatively frequently throughout the year, including in winter, in different regions of Poland (e.g., Kadulski 1977, Izdebska 2000, 2004, Karbowiak *et al.* 2003). A typical parasite among the bison of the Białowieża Forest also appears to be *Dermacentor reticulatus* (Fabricius 1794), which is commonly known as the marsh tick, the ornate cow tick, or the ornate dog tick, and which is common throughout this population also during winter (Izdebska 2004; Karbowiak *et al.* 2003). Until recently, *D. reticulatus* was considered to be a rare tick species in Poland (Szymański 1967), and was noted mainly in the northeast of the country (Dróżdż 1963; 1964; Dróżdż, Szymański 1965; Krzemiński 1968; Kadulski 1975; 1989; Szymański 1977; 1987; Siuda 1993; Bogdaszewska, Dróżdż 1997). Currently, this species is thought to be fairly widespread throughout the country, while it is a dominant species in the northeast (Siuda *et al.* 1997, Bogdaszewska *et al.*. 2006, Biaduń, Najda 2007, Kadulski, Izdebska 2009). Significantly, it is a frequent and numerous parasite of the bison in the Białowieża Forest, while it is noted sporadically among bison from other regions (Izdebska 2001a; 2001b; 2001c).

Both species of tick exhibit analogous topical specificity on different species of ungulates (Kadulski 1989; 1996). However, in bison, where they occur synhospitaly, *I. ricinus* is located similarly as it is on other large ungulates, while *D. reticulatus* exhibits alternative localization. Sheep ticks are usually noted in the inguinal regions, laterally on the body, and neck while the marsh tick is usually located at the tops of the pinnas on bison (Izdebska 1998; 2004). The occurrence of ticks on bison was studied mainly in the winter months, which was during the tick diapause period. The localization of *D. reticulatus* observed can be linked to its wintering behavior. It is, however, difficult to explain why ticks choose the pinnas as wintering sites since thermal conditions there are the least stable. Perhaps the Białowieska marsh ticks lengthen the fall period of activity and then remain in the location on the host in which they had fed. Proof of this were the distinct signs visible on the pinnas of the ticks having fed. It remains difficult to determine why the ticks chose this site to feed. Hematophagous parasites prefer areas on hosts that have thin skin that provides ready access to circulatory system vessels, stable environmental conditions, and low likelihood of mechanical removal (e.g., when the host animals clean themselves). In the case of march ticks that occurred on the pinnas of the bison, the most likely of these reasons appears to be the ready availability of food. It is difficult, however, to consider this based on the few publications that focus on bison skin structure and coat cover since these do not include analyses of skin thickness in these areas (Sokolov 1962; 1979).

The areas of bison skin included in the current study were chosen because either ticks were noted in them or there were distinct changes on the skin that indicated that ticks had fed there previously. The changes resulting from the feeding of various tick species were noted and the bison skin thickness in correlation with the sizes of the ticks' mouth parts was analyzed.

Materials and methods

Sixty samples taken from the skin of 10 adult bison, *Bison bonasus*, (5♂ and 5♀) were analyzed in the 2000–2004 period in the Białowieża Forest. The skin samples, which were taken from areas on the bison where ticks had been noted or where changes caused by tick bites were confirmed macroscopically, were frozen and then fixed in Bouin's solution. Histopathological changes were analyzed on 278 material samples that had been prepared according standard methods and stained with hematoxylin and eosin.

Additionally, skin thickness measurements were taken on 30 bison (15♂ and 15♀), and, to allow for comparisons, measurements of skin thickness in analogous regions were taken in red deer, *Cervus elaphus*, which is and alternative host for the same species of tick.

The ticks were collected and prepared according to methods that are recognized as standard for investigations of mammalian ectoparasites (Kadulski, Izdebska 2006). Permanent preparations in polyvinyl lactophenol were made of some specimens, and elements of the gnathosoma, which is used to penetrate the tissues of the host, were measured (Fig. 1).

Results and discussion

It was interesting that *D. reticulatus* was noted feeding and undergoing diapause only on the tops of the pinnas on bison, while *I. ricinus* preferred other locations (abdomen, in inguinal regions, legs, side of the body, the neck, and if the pinnas, then only at the base of them) (Izdebska 2004), which are analogous in other species of ungulates (e.g, Kadulski 1989). To explain the different topical specificity of these ticks, bison skin samples from the sites where ticks were observed were analyzed. The histological pictures of the preparations were used to analyze the changes that occurred in the skin as a result of tick feeding. Then comparisons were made of the thickness of skin in various bison body areas and the gnathosoma dimensions of the tick stages of both species observed feeding on these ungulates. Analogous measurements were taken of the skin of red deer, which is also a typical host for these ticks.

Observations of the picture of changes in bison skin after *I. ricinus* feeding

Tick feeding begins with the choice of a suitable location on the host, cutting the skin with the chelicerae, and then inserting the hypostome into the wound at the appropriate angle (Binnington, Kemp 1980; Buczek 2002; Siuda 1991). During feeding, the wound gradually develops a hematoma; necrosis and inflammation also occur in the host's skin which leads to the formation of a cavity filled with morphotic elements, erythrocytes, and leukocytes from the damaged blood vessels. The tick salivary glands play a significant role here since they excrete an anticoagulant that helps the ticks to feed on blood. External skin changes that result from ticks feeding are usually local in character and usually present as skin redness and swelling. The occurrence of histopathological changes is linked to the immune response of the host to the damage caused to the skin and blood vessels and also by the introduction of saliva and pathogens into the wound (Ribeiro 1987; Deryło, Buczek 1980; Piotrowski 1990; Buczek *et al.* 2000; Buczek 2002; Izdebska, Rolbiecki 2006).

Samples of skin that showed evidence of *I. ricinus* infestation were taken from near the anus, the inguinal areas, the abdomen, and legs. Varying degrees of histopathological change were noted from small areas with inflammation with small quantities of leukocytes to larger areas of inflammation that reached into the dermis. Inflammation reached from the outer surface of the skin, through to the epidermis where it developed into greater infiltration with large quantities of leukocytes in the dermis. Histopathological changes were confirmed on the slide preparations as the congregation of leukocytes (inflammation) that were visible in the streaks that extended from the edge of the epidermis into the dermis. The most changes were noted in preparations made of samples from bison inguinal regions and the legs. Of the 64 preparations made with samples from these areas, changes were confirmed in 39 (61%). Sixty-five preparations were made with samples from the abdomen area, and inflammation was confirmed in 35 (54%) of these, while in the 59 preparations made from the anal area, 19 (32%) were confirmed to exhibit inflammation.

It is precisely in these areas that external changes were observed previously and identified as having been caused by tick bites (redness and swelling) (Izdebska 2004; Izdebska, Rolbiecki 2006).

The degree of skin changes and the composition of the infiltration depended on the species, the length of the feeding period, the developmental stage of the tick as well as on the host species and its immunoresistance. Changes in the histopathological picture during the particular phases of feeding were a response to the introduction of active biological components into the wound, but they also occurred as an allergic response to the components of tick saliva. One of the consequences of feeding was the occurrence of acute inflammatory hemorrhagic syndrome (Ribeiro 1987; Piotrowski 1990; Siuda, Siuda 2005). Similar changes were observed following infestations of ticks of the genus *Ixodes* on the skins of rabbits, guinea pigs, and mice (Deryło, Buczek 1980; Deryło 1986; Buczek *et al.* 2000; Buczek 2002). On the first day after the ticks fed, the infiltrations were filled with leukocytes, among which neutrophil granulocytes and limphocytes dominated, and in the following days these came to be dominated by eosinophil granulocytes, acidophils, and lymphocytes, and less frequently by basophil granulocytes or monocytes. Large quantities of erythrocytes, which impact the damages vessels, were also confirmed (Deryło, Buczek 1980; Buczek *et al.* 2000; Buczek 2002).

In the current study, the pictures of changes in bison tissues were consistent with various stages of pathogenesis.

Observations of the picture of changes in bison skin after *D. reticulatus* feeding

Ninety preparations were prepared from samples taken from the pinnas of bison, of these 65 (72%) presented with distinct changes following feeding by *D. reticulatus*. The pictures were similar to those following feeding by *I. ricinus*, e.g. progressive inflammation that presented as leukocyte streaks that ran from the epidermis to the dermis. The character of the changes themselves was less severe, since no infiltrations were noted in the pictures. Similar observations were noted following feeding by *D. reticulatus* on samples of pinna skin from bison analyzed in previous studies (Izdebska, Rolbiecki 2006).

Analysis of reasons for different topical specificity in different tick species infesting bison

Measurements of gnathosoma length were taken in both of the tick species investigated. Morphometric analysis of the hypostoma of adult *I. ricinus* and *D. reticulatus* ticks (30 specimens from each species) indicated that the hypostoma length proportion of the former species in comparison to the latter was respectively 1.6–1.7 : 1 (Tabl. 1, Fig. 1)). This is likely why *D. reticulatus* chose the pinnas of the bison for their feeding site (and then the diapause site) since the skin of this area is very thin and the blood vessels are located just beneath it. Unfortunately, no measurements have been made to date of bison pinna skin thickness, nor is there much data regarding the skin thickness of other body parts of these ungulates. Some papers report that the thickest skin is on the back, neck, and the area around the tail, while thinner skin is in the inguinal areas and on the rear pasterns. This is why skin thickness measurements were taken in different areas of the body on adult bison. These data were compared to analogous measurements from red deer (Tabl. 2). Distinct differences were noted in the skin thickness between bison and red deer in areas that are considered to be typical infestation sites for *D. reticulatus* in different ungulates. In these areas the skin of red deer is thin, which guarantees access to blood vessels even for ticks with short gnathosoma. The skin of bison in analogous areas is substantially thicker, and the only skin that is suitable for penetration by *D. reticulatus* appears to be that on the pinna.

Figure 1. Gnathosoma of ticks: A – *Dermacentor reticulatus*, B – *Ixodes ricinus*

Table 1. Gnathosoma length of ticks [in mm] including stages of both species observed feeding on the large ungulates

Species of ticks	***Dermacentor reticulatus* male [n=60]**	***Dermacentor reticulatus* female [n=60]**	***Ixodes ricinus* female* [n=60]**
Length of gnathosoma	**0.65** ±SD 0,07	**0.61** ±SD 0.06	**1.03** ±SD 0.06

* males of *I. ricinus* are found on hosts, but they do not suck blood

Table 2. Measurements of the skin thickness [in mm] of the European bison and the red deer

Location	**Leg (pastern)**	**Inguinal region**	**Abdomen**	**Pinna**
Mean thickness of European bison skin [n=30]	**3.48** ±SD 0.37	**4.33** ±SD 0.25	**5.09** ±SD 0.13	**1.58** ±SD 0.06
Mean thickness of red deer skin [n=30]	**1.55** ±SD 0.49	**1.40** ±SD 0.42	**1.34** ±SD 0.45	**2.31** ±SD 1.12

Conclusions

Tick evolution was linked to the radiation of placental mammals and birds, which provided these parasites with many new niches. The formation of host specificity is shaped by substantial adaptation to host hemostatic system and limitations posed by the host immunological system (Mans *et al.* 2003, Mans and Neitz 2004). Ticks do, however, exhibit distinct topical specificity during feeding. While the reasons for topical specificity vary, it does appear that in the case of *D. reticulatus* a significant factor is the correlation between the length of the gnathosoma and the skin thickness of the host, which determines the availability of the circulatory vessels.

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Kleszcze (Acari, Ixodidae) jako pasożyty żubrów – przyczyny zróżnicowanych preferencji topicznych

Streszczenie: Kleszcze (Ixodidae) to obligatoryjne, hematofagiczne roztocze pasożytnicze kręgowców lądowych, notowane także u żubrów. Są najczęściej oligo-lub polikseniczne, niewiele jednak wiadomo mechanizmach kształtowaniu się ich specyficzności żywicielskiej i topicznej Kleszcze wykazują zazwyczaj wyraźne preferencje w wyborze lokalizacji u żywicieli. U ssaków kopytnych stwierdzane były zazwyczaj w pachwinach, na brzuchu, boku ciała, czy na szyi. Jednak *D. reticulatus* u żubrów zasiedla głównie wierzchołki małżowin usznych. Trudno wyjaśnić przyczyny takiej lokalizacji, gdyż jest to miejsce o wyjątkowo niestabilnych warunkach termicznych. Wydaje się, że pasożyty hematofagiczne preferują przede wszystkim miejsca o cienkiej skórze, czyli łatwym dostępie do naczyń krwionośnych, stabilnych warunkach środowiska, czy małych możliwościach usunięcia mechanicznego (np. w wyniku samooczyszczania się żywiciela). W wypadku kleszczy łąkowych u żubrów najbardziej prawdopodobną z wymienionych przyczyną lokowania się na wierzchołkach uszu wydaje się zatem łatwy dostęp do źródeł pokarmu.

Obecnie porównano grubość skóry żubrów i jeleni z miejsc preferowanych przez kleszcze pospolite i łąkowe, z rozmiarami narządów gębowych tych kleszczy uwzględniając stadia żerujące na dużych ssakach kopytnych (samice *I. ricinus* oraz samce i samice *D. reticulatus*). Stwierdzono, że gnathosoma adulti kleszczy łąkowych jest ok. 1,6–1,7 razy krótsza, niż samic kleszczy pospolitych. Rozmiary gnathosomy obu gatunków pozwalają na penetrację skóry jeleni w obrębie różnych rejonów tułowia, czy kończyn. Jednak skóra analogicznych okolic ciała dorosłych żubrów jest znacznie grubsza. Zmusza to kleszcze do szukania odpowiednich do żerowania miejsc, gdzie mają zapewniony dostęp do naczyń krwionośnych. I tu więcej możliwości stwarza dłuższa gnathosoma *I. ricinus*, podczas gdy najdogodniejszą lokalizację dla *D. reticulatus* stanowią małżowiny, gdzie skóra jest cienka i dobrze ukrwiona.