SHORT COMMUNICATION

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A unique phenomenon of oral-anal contact between ticks observed in two tick species *Ixodes ricinus* and *Dermacentor reticulatus*

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Abstract

For the first time in the world literature, the presented study describes oral-anal contact between two sympatric *Ixodes ricinus* and *Dermacentor reticulatus* ticks, which are the vectors of many pathogens in Europe. Among the specimens collected in nature, an *I. ricinus* male was found with the hypostome and chelicerae inserted in the anal aperture of a *D. reticulatus* female. Given the biological and physiological traits of both species and microorganisms colonising these arthropods, it seems that the interspecific oral-anal contacts between ticks may be an unknown alternative route of transmission of pathogenic and symbiotic microorganisms present in ticks' alimentary tract, faeces, body surface, and in the haemolymph and organs, taking place when the male's mouthparts are inserted into the female's body. The authors suggest that interspecific contacts between ticks in natural conditions can contribute to the spread of some microorganisms among different tick species and their hosts.

Key words

Ixodes ricinus, *Dermacentor reticulatus*, tick behaviour, oral-anal contact between ticks, tick-borne pathogen transmission, mechanical transmission

INTRODUCTION

Although many studies have been conducted to-date in laboratory and field conditions in many regions around the world, the behaviour of ticks at different stages of their life cycle has not yet been fully explored. Since these arthropods spend the greater part of their lives in the environment, research on their biology and physiology in the non-parasitic phase of the life cycle is particularly important. Such investigations may contribute to progress in the elucidation of many phenomena associated with e.g. tick spread and survival in the environment, and their involvement in the transmission of microorganisms, including pathogens, in nature.

This study is the first report in the world literature of oral-anal contact between two ticks from two species: an *I. ricinus* male and a *D. reticulatus* female in the non-parasitic phase of their life cycle. So far, this type of physical contact between two individuals has not been documented, even in ticks of the same species. Particularly interesting is this type of research conducted on species with great epidemiological significance, such as *Ixodes ricinus* and *Dermacentor reticulatus*. These tick species have a wide distribution range [1, 2] and a broad spectrum of hosts, including the same species of wild living and domestic animals [3, 4]. *I. ricinus* ticks also are frequent parasites of humans [5, 6], whereas *D. reticulatus* ticks attack humans more rarely [4, 5, 7]. The presence of *I. ricinus* and *D. reticulatus* ticks in the same areas, or even in the same habitats [3, 8, 9] and infestations of the same hosts promote pathogen transmission in various conspecific and interspecific biological systems.

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MATERIALS AND METHOD

On 6 May 2017, hungry adult *I. ricinus* and *D. reticulatus* specimens were collected from vegetation by the flagging method in a common habitat of the two species in eastern Poland (51°27'N 23°10'E). The electronic devices used during the field study recorded a temperature of 23.8 °C and 49% humidity in the locality. The specimens collected on the cloth during 1-h collection were placed together in a rearing chamber and transported to the laboratory. Prior to the viewing and segregation of the collected specimens according to the species, gender, and developmental stage, the chamber was kept in the dark in a refrigerator at a temperature of approx. 5 °C and approx. 80% humidity. After about 20 h post-collection, two attached tick specimens of the different species, i.e. *I. ricinus* and *D. reticulatus*, were observed during the morphological analysis. Every consecutive day at the same time (ca. 11.00 a.m.), i.e. at the time of high diurnal activity of the two species in eastern Poland [10], the ticks were transferred to a Petri dish and viewed under an Olympus SZX16 stereoscopic microscope in a room with a monitored temperature of 22 °C and approx. 50% humidity. The observations were continued until detachment of the specimens from each other.

Each time, the ticks were touched with tweezers and a preparation needle to check whether they were still attached. Next, to assess the degree of attachment of the hypostome of one tick in the anal opening of the other, the attached specimen was grasped with the tweezers as close as possible to the gnathosoma and pulled gently.

RESULTS AND DISCUSSION

Among the 71 *I. ricinus* specimens (22 females, 21 males, and 28 nymphs) and 20 *D. reticulatus* ticks (16 females and 4 males) collected in the habitat, one unengorged *I. ricinus* male was noted attached to an unengorged *D. reticulatus* female. Morphological examinations confirmed that the hypostome and chelicerae of the *I. ricinus* male were inserted in the anal aperture of the *D. reticulatus* female (Fig. 1). The hypostome ensured strong attachment of the male to the body of the female. The ticks remained in the same position for 8 days. Even such stimuli as moving the ticks with the tweezers and preparation needle, illumination with an intense light beam while viewing and taking photographs of the specimens, a rise in the ambient temperature (from 5 °C in the refrigerator to 22 °C in the laboratory), and the gentle pulling of the *I. ricinus* male gnathosoma while checking the attachment strength, did not stimulate these species to detach.

Under the influence of carbon dioxide exhaled by the researchers, i.e. potential hosts, and their smell, as well as the rise in the temperature and changes in ambient humidity, which generally influence tick host seeking behaviour [10–14], the *D. reticulatus* female with the attached *I. ricinus* male moved quickly around the Petri dish. The intensified locomotor activity of the female did not alter the degree of attachment of the hypostome of the *I. ricinus* male in its anal opening (Fig. 2a, 2b). Not until the 8th day of observations did the *I. ricinus* male withdraw its mouthparts from the anal opening of the *D. reticulatus* female, but it did not leave the female and did not change its position. Its palpi, chelicerae, and hypostome touched the female's perianal region and the legs still strongly embraced its idiosoma (Fig. 2c, 2d).

No literature reports have been found on a similar case of the attachment of a male's mouthparts in the female's anal opening. This phenomenon is even more interesting in the light of the fact that the specimens represented two clades – Prostriata and Metastriata – which differ considerably in their mating strategies. In *I. ricinus* (Prostriata), sexual contact between adult males and conspecific adult females can take place both out of the host, i.e. on soil or plants, and during feeding on a host. Blood meal ingestion in the case of these ticks is not necessary for the induction of courtship and mating, which involve male attraction, moulting, and copulation. In contrast, mating in *D. reticulatus* (Metastriata) takes place exclusively on the host. Spermatogenesis in these ticks starts after the beginning of male feeding. Males of this species react to volatile sex pheromones produced by feeding conspecific females only after ingestion of a small blood portion or when engorged.

The reasons for the behaviour of the *I. ricinus* and *D. reticulatus* ticks observed in this study are difficult to explain. The interspecific several-day long oral-anal contact between the ticks indicates that the compounds contained in the faeces and/or the cuticular substances in the *D. reticulatus* female strongly induced the attraction and attachment behaviour of the *I. ricinus* male. The behaviour of the *I. ricinus* male described in this study may also be associated with changes in the perception of external chemical or physical stimuli by the male receptors.

In the faeces of various tick species, similar compounds [15, 16] stimulating aggregation responses between conspecific specimens of argasid [15, 17] and ixodid ticks [16, 18] have been identified, i.e. guanine, xanthine, and uric acid, and

Figure 1. Oral-anal contact between *Ixodes ricinus* male and *Dermacentor reticulatus* female on 3rd day of observation. Ventral side view, the hypostome and chelicerae of the *I. ricinus* male inserted in the anal aperture of the *D. reticulatus* female

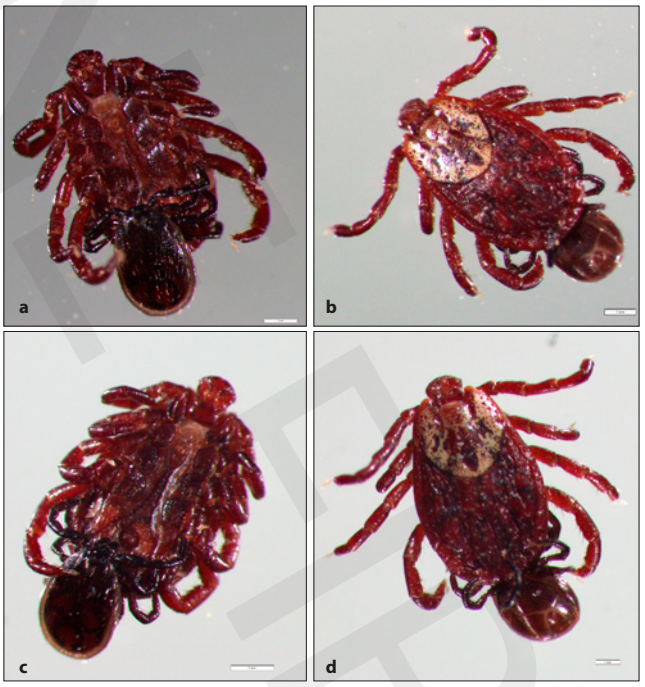

Figure 2a, 2b, 2c, 2d. Oral-anal contact between *Ixodes ricinus* male and *Dermacentor reticulatus* female. 2(a) and 2(b) – 7th day of observation, ventral and dorsal side of ticks: the hypostome and chelicerae of the *I. ricinus* male are still inserted in the anal aperture of the *D. reticulatus* female. 2(c) and 2(d) – 8th day of observation, ventral and dorsal side of ticks: the *I. ricinus* male withdrew its mouthparts from the anal opening of the *D. reticulatus* female but its palpi, chelicerae, and hypostome touched the female's perianal region and the legs still strongly embraced female's idiosoma.

some investigations demonstrated that mixtures of these compounds induced arrestment of adult *I. ricinus* ticks more strongly than their individual components [16]. Many components with the same compounds present at different concentrations have also been detected in cuticular extracts from Prostriata ticks, i.e. *I. ricinus* [19], and *I. scapularis* [20], and from Metastriata ticks, i.e. in some species from the genera *Dermacentor*, *Hyalomma, Rhipicephalus*, and *Amblyomma* [13].

Interestingly, the *I. ricinus* male observed in the current study reacted strongly to the pheromones of the female from another species, despite the presence of as many as 22 conspecific females in close proximity. The duration of the insertion of the mouthparts of the *I. ricinus* male in the *D. reticulatus* female anal opening was similar to the length of mating between *I. ricinus* males with conspecific females, which may take up to a week or even longer (own observations).

The changes in tick physiology affecting the cuticular chemistry in *D. reticulatus* females and/or the sensitivity of *I. ricinus* males to volatile compounds emitted by an interspecific female can also be caused by environmental conditions (thermal shock induced by the transfer of the ticks from a temperature of 23.8 °C in the natural environment to ca. 5 °C in the refrigerator or changes in the humidity). These hypotheses can be supported by the results of previous research conducted by other authors. Estrada-Peña [21] found alterations in cuticular hydrocarbons in *Rhipicephalus sanguineus* ticks from Northeast Spain, which, as suggested by the author, may have been induced by climatic conditions. Extreme temperatures correlated positively with six compounds, mainly methyl alkanes, identified in the cuticular hydrocarbon mixtures. The composition of cuticular hydrocarbons may differ between ticks from different populations [19, 21, 22]; therefore, differences in the aggregation, attraction, and attachment behaviour of ticks from the same species present in different localities of their occurrence range cannot be ruled out.

Tick-borne pathogens are also able to affect the host-seeking behaviour of ticks. Alekseev et al. [23] reported the disappearance of the activity of *Ixodes ricinus* nymphs and adults infected with *Borrelia* spirochetes, whereas non-infested ticks exhibited locomotor activity. Adult *I. scapularis* tick infected with *Borrelia* spirochetes were less active and less able to move vertically; they also exhibited host-seeking activity at lower heights than non-infected specimens [24]. In turn, other bacteria, *Arsenophonus* and *Rickettsia*, exert an effect on the locomotive ability of larvae of three tick species, i.e. *Amblyomma americanum*, *Dermacentor variabilis*, and *I. scapularis*. Tick motility was found to increase during *Rickettsia* infection and decrease during *Arsenophonus* infection [25].

It is unclear whether the phenomenon of the oral-anal contact in the ticks described in this study occurs frequently in natural conditions, or is only incidental. In previous tick collections from the authors region of eastern Poland which were maintained in the same conditions as in the presented study, an *I. ricinus* male with a gnathosoma adjoining the perianal area of the body of a *D. reticulatus* female, strongly embracing the female with its legs, was observed several times. Presumably, in some environmental conditions, sympatric tick species with similar patterns of seasonal activity enter into physical contact.

Although microbial transmission among ticks usually takes place via different routes, it seems that their oral-anal contacts can play a certain role in the spread of some pathogens, symbiotic bacteria, and fungi associated with these arthropods in nature. During such contact, the male can be infected with microorganisms present in the alimentary tract or faeces of the female, wherefrom the male can transfer them on its mouthparts onto a non-infected conspecific female during copulation or on to host skin during feeding.

The interesting and yet unreported phenomenon of the oral-anal contact between ticks requires further research to explain not only the frequency of its occurrence, but also its biological and perhaps epidemiological importance in natural conditions.

Competing interests

The authors declare that they have no competing interests.

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