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ORIGINAL ARTICLE

Conspecific hyperparasitism in the *Hyalomma excavatum* tick and considerations on the biological and epidemiological implications of this phenomenon

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Abstract

Objective. This study presents for the first time a case of *Hyalomma excavatum* hyperparasitism and an analysis of this phenomenon in terms of its potential role in the biology of ticks and epidemiology of tick-borne diseases.
Materials and method. Two partially engorged *H. excavatum* females, one fully engorged female, and 5 males were collected from a naïve rabbit and placed together in a rearing chamber at a temperature of 25 °C and 75% humidity.
Results. 3–4 days after tick detachment from the host's skin, one partially engorged *H. excavatum* female was observed attached to the idiosoma of the fully engorged conspecific female.
Conclusions. This study and observations of other authors confirm that partially engorged ixodid ticks can re-infest the host, and even co-feeding fully engorged ticks in order to collect the blood meal that is indispensable for important physiological processes. However, inefficient feeding of a partially engorged female on another conspecific female may reduce its reproductive performance and disturb the development of eggs and larvae. It seems that parasitism of a tick on another conspecific specimen, when at least one of them is infected by a microorganism, may be a yet poorly explored route of transmission of pathogens or symbionts between the ticks. Initiation of feeding by a hungry or partially engorged tick on a fully engorged specimen is an attempt to obtain food in the drastic conditions of the absence of a target host. Tick hyperparasitism with concurrent pathogen transmission can contribute to the genospecific diversity of pathogens in vectors and hosts.

Key words
ticks, *Hyalomma excavatum*, hyperparasitism, transmission of pathogens, tick feeding

INTRODUCTION

Ticks have a remarkable ability to adapt to various environments in the globally changing geoclimatic conditions, which contributes to the expansion of their distribution range. The increasing occurrence of ticks is accompanied by a growing risk to human and animal health posed by tick-borne diseases [1] and, hence, greater economic losses and costs [2, 3, 4]. Therefore, investigation of the biology of ticks and transmission routes of tick-borne pathogens is gaining importance.

Ticks are blood-ingesting parasites of terrestrial vertebrates, including humans. Ingestion of a blood meal by consecutive developmental stages is a prerequisite for tick survival and further development. The major mode of blood collection by ticks involves questing for a host, followed by successful feeding. Ticks are distinguished from other blood-ingesting arthropods by a variety of bioactive compounds secreted with saliva during feeding, which facilitate ingestion of a qualitatively and quantitatively sufficient meal during short- (Argasidae nymphs and adults) and long-term (Ixodidae) feeding [5], and ensure effective pathogen transmission [6].

Besides infesting their vertebrate hosts in order to collect blood, hungry ticks can also attack engorged conspecific or interspecific specimens. This phenomenon, called hyperparasitism, was described in ticks already at the end of the 19th century by Barber [7], who found an *Amblyomma variegatum* male (identified by the author as *Hyalomma venustrum*) parasitizing a female from the same species. In the family Argasidae, hyperparasitism was noted mainly in representatives of the genus *Ornithodoros*. In turn, in the family Ixodidae, the phenomenon was reported from several species of the genus *Ixodes* (prostriata ticks), as well as genera *Amblyomma*, *Aponomma*, *Rhipicephalus* (*Boophilus*), *Dermacentor*, *Hyalomma*, and *Rhipicephalus* (metastriata ticks) (Tab. 1).

Since many viruses, bacteria, protozoa, and even some microfilaria, are transmitted with tick saliva secreted during the feeding process [1, 3, 8], it seems possible that parasitism of one tick on another may play a role in the spread of pathogens in the environment. The fact that conspecific

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Table 1. Reports about cases of conspecific and interspecific hyperparasitism in ticks (Ixodida)

Hyperparasitic specimen and degree of engorgement	Host specimen and degree of engorgement	Distribution of tick species/habitat	Main animal hosts	References
Conspecific hyperparasitism				
Argasidae ticks				
Ornithodoros turicata[2] unengorged female	*Ornithodoros turicata*[2] fully engorged female	Southern part of North America, from Kansas south to central Mexico, with a disjunct population in Florida/ caves and ground squirrel or prairie dog burrows	Mammals, e.g. rodents that live in burrows, domestic pigs, cattle and other livestock; birds, e.g. owls; reptiles, e.g. snakes; tortoises (A, I);	Wood according to [28]
Ornithodoros turicata[2] (former *Ornithodoros turicata americanus*)	*Ornithodoros turicata*[2] former *Ornithodoros turicata americanus*)			
Ornithodoros turicata[1] starved female	*Ornithodoros turicata*[1] fully engorged male			Francis 1938 according to [28]
Ornithodoros turicata[1] unfed fourth stage nymph	*Ornithodoros turicata* engorged nymph			Francis 1938 according to [28]
Ornithodoros parkeri [1] unengorged last nymphal stage	*Ornithodoros parkeri*[1] fully engorged last nymphal stage	Southern USA and Latin America/various ecological habitats in caves and burrows of hosts	Mammals, e.g. ground squirrels and prairie dogs (A, I)	[66]
Ornithodoros parkeri[1] unengorged males	*Ornithodoros parkeri*[1] fully engorged female			[69]
Ornithodoros erraticus[1] males and nymphs that produced males	*Ornithodoros erraticus*[1] females and nymphs that produced females	North and East Africa, Near East, South-Eastern Europe/ burrows of pig pens or other habitats of hosts	Mammals, e.g. pigs, bovine, sheep, rodent, and rarely also birds (A, I)	[50]
Ornithodoros tartakowskyi[3]	*Ornithodoros tartakowskyi* [3]	Central Asia/ burrows of various rodents	Mammals: rodent species, but primarily the great gerbil, *Rhombomys opimus (A, I)*	[49]
Ornithodoros tartakowskyi[3]	*Ornithodoros tartakowskyi* [3]	Central Asia/ burrows of various rodents	Reptiles (A, I)	[8]
Ornithodoros (*Alectorobius*) *puertoricensis*[2]	*Ornithodoros* (*Alectorobius*) *puertoricensis*[2]		Small mammals, e.g. rats, and also amphibians, reptiles (A, I)	[68]
Ixodidae ticks (Prostriata ticks)				
Ixodes holocyclus[1] males	*Ixodes holocyclus* engorged females	Australia, mainly along coastal eastern area / areas of high rainfall, e.g. wet sclerophyll forest and temperate rainforest	Small mammals, birds; sometimes reptiles (I); mammals, mainly carnivores and ungulates (A, I)	[9]
Ixodes holocyclus[1] male	*Ixodes holocyclus*[1] female			[29]
Ixodes pilosus[3] males	*Ixodes pilosus*[3] females	Africa – tropical part/bush, humid habitats	Mammals, mainly carnivores and ungulates (A, I)	[30]
Ixodes trianguliceps[3] males	*Ixodes trianguliceps*[3] female	Euroasia/forests, hedgerows and heaths, open areas	Mammals, mainly insectivores and rodents	[31]
Ixodes (*Afrixodes*) *moreli* males	*Ixodes* (*Afrixodes*) *moreli* female		Mammals, mainly ungulates	[32]
Ixodes persulcatus[1] males *Ixodes angustus*[2] male	*Ixodes persulcatus*[1] females *Ixodes angustus*[2] engorged female	Euroasia, from Nord-Eastern Europe through Central and Northern Asia to the Peoples Republic of China, Taiwan and North Korea, and northern Japan/ usually mixed deciduous-coniferous forests, taiga Holarctic region, mainly northern latitudes or mountain regions of southern latitudes in North America, also Japan, and Eastern Russia/ cool, moist habitats, including forests (particularly coniferous tracts), and along the shores of rivers. Rarely found outside the nest of its host.	Small and medium-sized mammals, birds, and reptiles (I); Medium-sized and large mammals, mainly wild and domestic ungulates, hares and dogs (A) Small mammals, mainly rodents (voles, mice and the red squirrels) and shrews (I, A). Rarely feeds on humans or domestic animals.	[70] [73]
Ixodidae ticks (Metastriata ticks)				
Amblyomma variegatum[1] male [formerly *Hyalomma venustrum*]	*Amblyomma variegatum*[1] engorged female [formerly *Hyalomma venustrum*]	West-Central and Central Africa, Asia (Southern Arabia and several islands in the Indian Ocean); several islands in the ntic Ocean and Caribbean/ Savannah	Small mammals, ground-feeding birds, and reptiles (I); livestock, camels, antelope, and other wildlife ungulates (A)	[7]
Rhipicephalus (*Boophilus*) *annulatus*[3] unengorged female [formerly *Boophilus annulatus*]	*Rhipicephalus* (*Boophilus*) *annulatus* [3] (sex is unknown) [former *Boophilus annulatus*]	Africa, America, Asia and Australia, tropical and subtropical regions/ areas with canopied mesquite and mixed brush vegetation	Mammals, mainly undulates (cattle, buffalo, bison and cervids, e.g. deer, antelopes, etc.)	[71]

Hyperparasitic specimen and degree of engorgement	Host specimen and degree of engorgement	Distribution of tick species/habitat	Main animal hosts	References
Rhipicephalus (*Boophilus*) *annulatus*[3] unengorged male [formerly *Boophilus annulatus* (*Margaropus annulatus*)]	*Rhipicephalus* (*Boophilus*) *annulatus*[3] engorged female [formerly *Boophilus annulatus* (*Margaropus annulatus*)]			Hooker et al. 1912 according to [28]
Rhipicephalus haemaphysaloides pilans [1] partially engorged female [formerly *Rhipicephalus haemaphysaloides paulopunctatus* Neumann]	*Rhipicephalus* (*Boophilus*) *microplus*[3] fully engorged female [formerly *Boophilus microplus*]	*R. haemaphysaloides*: South-Eastern Asia (Nepal, Burma (Myanmar), India, Taiwan, Sri Lanka, China, Indonesia, Thailand, Vietnam) *R. microplus*: as mentioned above	*R. haemaphysaloides*: small mammals and birds (I); domestic mammals, e.g. cattle, buffalo, horse, sheep, camel, donkey, dog, and many wild mammals (A); *R.microplus*: as mentioned above	[28]
Hyalomma detritum [2] male (syn. *H. scupense*)	*Hyalomma detritum* [2] fully engorged female (syn. *H. scupense*)	Central and Southern Asia, Near East; Southern Europe; North-Western Africa/ humid areas in steppes, deserts, and semi-deserts	Mammals, mainly cattle and other ungulates (A, I)	[25]
Hyalomma detritum [2] (syn. *H. scupense*) unengorged nymph [formerly *Hyalomma mauritanicum*]	*Hyalomma detritum* [2] (syn. *H. scupense*) engorged nymph [formerly *Hyalomma mauritanicum*]			[24]
Amblyomma fuscolineatum [3] two males [formerly *Aponomma crassipes, Aponomma fuscolineatum*]	*Amblyomma fuscolineatum* [3] engorged female [formerly *Aponomma crassipes, Aponomma fuscolineatum*]	Continens: Australasia Oceania Melanesia (Papua New Guinea) America North America (USA)	Reptiles	Roubaud and Colas-Belcour 1935 according to [28]
Amblyomma rotundatum [2] unergorged female	*Amblyomma rotundatum* [2] partially engorged female	South and Central America (Argentina to Mexico, including the Caribbean Islands) and southern part of Florida / savannah, forest savannah and rainforest habitats	Reptiles and amphibians; birds (I, A)	[27]
Hyalomma excavatum [1] partially engorged female	*Hyalomma excavatum*[1] fully engorged female	Central and Sout-West Asia; Middle and Near East; South-Eastern Europe and southern Africa/warm arid and semi-arid *habitats*	Small mammals, mainly rodents that live in burrows, and birds (I); large mammals (A)	Buczek et al. present study
Interspecific hyperparasitism				
Ixodes sp. male	*Bothriocroton auruginans* female [formerly *Aponomma auruginans* Schulze]	Australia/ *I. sp.* as mentioned above *B. auruginans*: burrows in savanna or grassland, and forests	*B. a.* Mammals: e.g. wombats *Ix.* sp.	[9]
Ixodes sp. male	*Rhipicephalus (Boophilus) microplus* female [formerly *Boophilus microplus*]	*R. microplus*- as mentioned above	*R. microplus*: mammals, mainly ungulates and carnivores (A, I)	[9]

[1] parasitizing on humans; [2] occasionally parasitizing on humans; [3] parasitizing on animals (no literature data about feeding on humans exists); (A – adults, I – immatures).
* No literature data exists about feeding on humans.

and interspecific tick contacts have been observed not only in the laboratory but also in nature, prompts consideration of this route of transmission of pathogenic and symbiotic microorganisms among ticks [9].

This current study presents an interesting case of *Hyalomma excavatum* female parasitism on another conspecific female, and analyses the biological and epidemiological implications of tick hyperparasitism.

The *H. excavatum* tick is widespread in the Mediterranean region as well as steppe areas of North Africa and South Asia [10]. However, the predicted changes in temperature and precipitation rates will alter the distribution of various species, including *H. excavatum*, which differ in their predilection for habitat conditions [11].

Adult stages infest wild and domestic animals, mainly buffalo, cattle, sheep, goats, camels, horses, and donkeys [12, 13], but can also attack humans [14, 15]. Larvae and nymphs parasitize hares, hedgehogs, and rodents [10].

Since this tick was previously identified as *Hyalomma anatolicum* and can still be mistaken for this species due to the high morphological similarity, the competence of *H. excavatum* to transmit tick-borne pathogens is not known. *Theileria buffeli/orientalis*, *Babesia* spp., *Anaplasma/Ehrlichia* [16], *Coxiella burnetii* [17, 18], Spotted Fever Group Rickettsiae [19, 20], and Crimean-Congo Haemorrhagic Fever Virus (CCHFV) [21] were detected in *H. excavatum* removed from animals. CCHFV is spread in 30 countries and infections with this virus have a fatal outcome in as many as 50% of cases in humans [22]. Furthermore, *Borrelia burgdorferi* sensu lato and *Rickettsia aeschlimannii* were isolated from *H. excavatum* ticks collected from patients' skin [23].

To the best of the authors' knowledge, to date hyperparasitism has not been described in *H. excavatum*. In the available literature, only two publications were found on hyperparasitic ticks from the genus *Hyalomma*. Both papers focused on the same species, i.e. a *Hyalomma detritum* tick foraging on another conspecific specimen. An unengorged *Hyalomma mauritanicum* nymph (at present *H. detritum*) attached to an engorged nymph was found by Sergent [24] during investigations of the foraging of these ticks on a calf, and a *H. detritum* male attached to an engorged female of this species was observed by Usakov [25].

MATERIALS AND METHOD

H. excavatum rearing was started with specimens kindly donated by colleagues from the Institute of Zoology SAS in Bratislava in 2013. Since these ticks represent a two-host species (only occasionally a three-host species), they were fed on naïve New Zealand rabbits (*Oryctolagus cuniculus*) twice during their life cycle (adult and larval stages). In one investigation by the authors of the current study, research was undertaken of the biology of *H. excavatum*, in which larvae hatched from eggs laid by one female were placed on one host.

H. excavatum larvae were placed on 10 cm^2 of shaved rabbit skin. After the larvae had moulted into nymphs and the nymphs into adult stages, ticks that had detached from the host skin were collected and transferred to rearing chambers. The chambers were kept at a temperature of 25 °C and 75% humidity, maintained with the method proposed by Winston and Bates [26]. One chamber contained approximately 8 adult specimens, including 3 *H. excavatum* females with a varied degree of engorgement and 5 males.

All procedures performed on the animals were approved by the Local Ethics Committee (No. 22/2015).

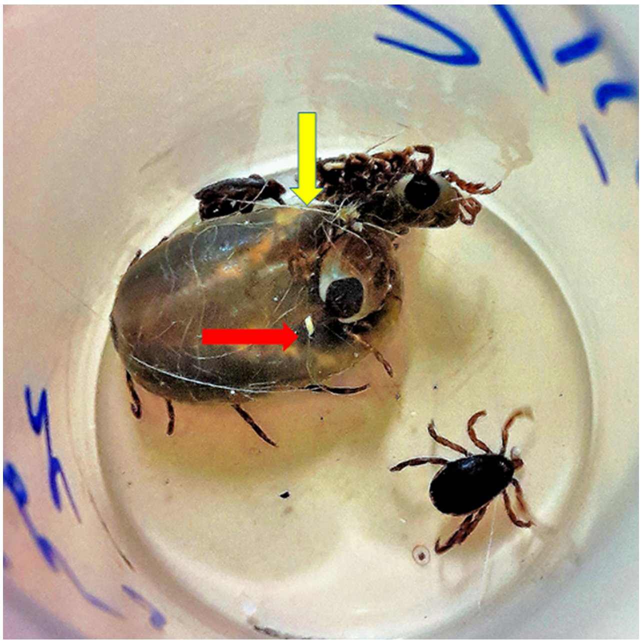

Figure 1. *Hyalomma excavatum* partially engorged female attached to the posterior dorsal part of the idiosoma of a fully engorged conspecific female (red arrow); trace of tick bite –scar (yellow arrow)

RESULTS AND DISCUSSION

In one of the rearing chambers, there were 2 partially engorged females, one fully engorged female, and 5 males among the *H. excavatum* adult specimens collected from the naive host. The 2 partially engorged females attempted to replenish the amounts of food ingested during the infestation of the rabbit. Approximately 3 and 4 days after detachment from the host's skin, they attached to the posterior dorsal part of the idiosoma of a fully engorged conspecific female. One of the partially engorged females detached after several hours, whereas the other one remained with its hypostome inserted into the body of the fully engorged female (Fig. 1). As can be seen, the long mouthparts of the partially engorged *H. excavatum* female penetrated deep inside the body of the fully engorged specimen. Its entire hypostome was attached to the idiosoma of the other female. It probably reached the intestine with its multiple blood-filled caeca and the haemocel cavity of the fully engorged specimen. Disruption of the continuity of their walls caused an outflow of the intestinal contents and haemolymph. This is indicated by the darker colouration of the idiosoma of the fully engorged specimen at the site of attachment of the conspecific female. A similar sign was visible on the idiosoma after the feeding of the other partially engorged female.

Hyperparasitism in ticks has been poorly explored. Hungry ticks attached to engorged specimens were usually found accidentally among ticks collected from animals or in laboratory rearing conditions. Therefore, it is not known how often and in which conditions ticks become hyperparasitic in nature. The few papers available provide exact numbers of ticks among which hyperparasitic specimens were found. In a group of 82 *Amblyomma rotundatum* ticks (81 females and one nymph) removed from snakes, Labruna et al. [27] found one unengorged female attached to a partially engorged female already 15 minutes after removal from the host.

Tick hyperparasitism may not be as rare in nature as suggested by data provided by some authors [24, 28]. This is indicated by multiple traces of tick bites (scars) that were found on the idiosoma of engorged specimens representing prostriata ticks [9, 29–33], and metastriata ticks [27] that were close to other hungry or partially engorged conspecific specimens. In ther opinion of the authors of the current study, the dark-coloured permanent scars on the tick idiosoma and some exoskeletal anomalies in specimens collected in nature [34–37] may have been caused by other ticks that parasitized these specimens, or their earlier stages, i.e. nymphs (own observations, unpublished data).

As many as 27.8% of 162 *Ixodes trianguliceps* females examined were found to have been attacked by conspecific males. The number of scars on the female bodies increased with the higher degree of engorgement. Scars were noted in 41% of partially fed females. The female genital opening was the most common site of attachment of males [31].

One specimen can be parasitized by several other individuals. In *Ixodes (Afrixodes) moreli* females, 1 – 9 scars were noted. One female of this species was simultaneously parasitized by up to 5 males. Approximately 32.4% of *I. (A.) moreli* males attached to females [32], which may indicate a high frequency of this phenomenon.

Scars left by male tick bites on the bodies of *Ixodes persulcatus* females collected in nature were observed more frequently during hotter seasons than during seasons with moderate temperatures [33].

In certain circumstances, hyperparasitism can be the only way to obtain food by hungry tick specimens. In natural conditions, questing for a host by some species may be difficult, as they react to stimuli emitted by the host when present at a short distance from the source, e.g. *Argas reflexus* responds at a distance of a few cm from the host [38]. Moreover, non-nidicolous ticks with an ambush host-seeking life strategy, e.g. *Dermacentor reticulatus, Ixodes scapularis*, or *Ixodes ricinus,* cover short distances.

Over 7 weeks, *D. reticulatus* females and males at an average temperature of 18.3 °C and 56.7% humidity in the habitat, moved horizontally over a distance of only 66.35±100 cm and 54.85±45 cm, respectively [39]. *I. scapularis* adult stages cover a distance of 5 – 6 m over 3 – 4 weeks [40], and *I. ricinus* females and males move for a distance of ca. 4.75 m and 1 m, respectively, over 3 weeks [41].

A completely different situation occurs in *Hyalomma asiaticum*. It is reported that in this species 10% – 25% of marked ticks, one month after discharge were registered at a distance of 100 – 400 m; several individuals even more than 500 m. This is explained by the fact that while most tick species of genera *Ixodes* and *Rhipicephalus* passively wait for the host, *H. asiaticum* ticks actively search the host [42].

Hungry ticks usually attack partially or fully engorged specimens from the same species, but can also parasitize interspecific ticks (Tab. 1). In the current study, a partially engorged *H. excavatum* female was noted attached to a fully engorged female. This particularly interesting case shows that partially engorged ticks can repeatedly feed on fully engorged specimens. They can also quest for a new host, e.g. an animal or even a human, to replenish the amounts of food that will ensure maintenance of all vital functions and, possibly, their further development. A case of attachment of a partially engorged *I. ricinus* female to the forearm of a man has been noted. The tick detached from the skin of a cat (*Felis catus*) wearing an anti-tick collar. The tick fed on the patient for ca. 10 hours and infected him with *Borrelia burgdorferi* s.l. spirochetes, which was manifested by the development of erythema migrans (unpublished data). In the current study, partially engorged nymphs of *I. ricinus* were sampled several times by flagging methods (unpublished data). This means, that ticks need to take some critical portion of blood before metamorphosis (unpublished data).

Earlier, Anastos [28] described a partially engorged *Rhipicephalus haemaphysaloides paulopunctatus* female attached to a fully engorged male from a different species, i.e. *Rhipicephalus* (*Boophilus*) *microplus*. The specimens were found among several thousand ticks collected from domestic animals in the Dutch East Indies (today Indonesia).

Hard ticks usually attach to their host and detach only after ingestion of the maximum portion of a blood meal. The body weight of fully engorged specimens is several-fold higher than that of hungry ticks; it increases 190-fold in *I. ricinus* females, and 88-fold in *D. reticulatus* females [43]. Detachment of partially engorged *H. excavatum* females from the host may be caused by a high concentration of ticks, which triggers a strong local reaction in rabbit skin to the components of tick saliva introduced during feeding and changes in the quality of ingested blood meal.

The greater the intensity of tick invasion, the greater the changes in the parasitic phase parameters, such as the length of time and effectiveness of the feeding [43]. The weight of engorged females can be influenced by other factors, e.g. nutritional status of the host, host immunisation acquired in previous tick infestations, other species co-feeding on the same host [44–46], and some chemicals used for tick control in animals [47]. In natural conditions, the detachment of hungry and partially engorged ticks can be caused by the death of the host [48]. The amount of blood ingested by a female determines the number of oviposited eggs and, consequently, the number of offspring.

Different tick developmental stages, such as adult stages (males and females) and nymphs, can be hyperparasites (Tab. 1). Attachment of a hungry or partially engorged tick to an engorged specimen may be a desperate way of obtaining food in the absence of a target host, or a way of replenishment of food reserves by specimens detached from host skin before ingestion of a full portion of blood. Feeding, which involves alternate insertion of saliva and sucking host blood portions, promotes transmission of microorganisms from an infected to non-infected specimen, or even an exchange of pathogens between two infected ticks. During host blood ingestion, microorganisms present in the infected tick multiply intensively and spread with the haemolymph around the organism [49]. In a systemic infection, pathogens are present in many tick organs which can be penetrated by the mouthparts of another specimen. The long mouthparts of species from the genera *Ixodes*, *Aponomma*, *Amblyomma*, and *Hyalomma* are able to reach deep into the tick's body. Similarly, for example, Řeháček [50] wrote that during the generalized form of tick infection, rickettsiae were registered in haemolymph, intestine, salivary glands, Malpighi glands, ovaries, or testes of ticks, i.e. in the whole body of the tick.

Conspecific hyperparasitism may contribute to the spread of pathogens within a tick population in natural conditions. This is suggested by the results of previous laboratory investigations confirming transmission of *Dipetalonema viteae* in *Ornithodorus tartakovskyi* [51], *Borrelia crocidurae* in *Ornithodoros erraticus* [52], and *Borrelia burgdoreri* s.l. spirochetes in *Ixodes persulcatus* [33]. Furthermore, symbiotic bacteria or fungi whose biological role is not fully known can be transmitted during physical contact between ticks [53–55]. Many symbiotic microorganisms that turned out to be human and animal pathogens have been identified in ticks [56]. It seems that tick hyperparasitism can contribute to the high genospecific diversity of some pathogens, for instance *Borrelia burgdorferi* s.l. [57] or Crimean-Congo haemorrhagic fever virus [58], as well as multiple pathogen coinfections in ticks [59–64] and in hosts [65–67].

The current study indicates that partially engorged ixodid ticks can re-infect a host in order to accumulate an amount of food indispensable for their survival and further development. Hyperparasitism may be a yet poorly known route of microbial transmission in ticks, which may contribute to the spread and maintenance of pathogens in nature. It is probably responsible for the occurrence of genospecific diversity of pathogens in ticks and their hosts. Future investigations of the epidemiology of tick-borne diseases in humans and animals should consider this route of pathogen transmission and explain its role in natural conditions.

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