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Przebieg żerowania kleszczy *Ixodes ricinus* (Ixodida: Ixodidae) w odniesieniu do transmisji patogenów

Streszczenie

Wstęp. W Europie gatunkiem kleszczy najczęściej pasożytującym na człowieku jest kleszcz pospolity – *Ixodes ricinus*. Jego największe medyczne znaczenie wiąże się z przenoszeniem bakterii *Borrelia burgdorferi* sensu lato i *Anaplasma phagocytophilum*, riketsji gorączek plamistych, wirusów kleszczowego zapalenia mózgu i pierwotniaków Babesia spp., którymi zakaża żywiciela zazwyczaj podczas żerowania.

Cel. Celem pracy było opisanie przebiegu żerowania kleszcza pospolitego – *Ixodes ricinus* oraz przeanalizowanie czynników wpływających na zdolność tego gatunku do transmisji patogenów.

Materiał i metody. Przebieg żerowania dorosłych postaci *Ixodes ricinus* obserwowano na królikach w temperaturze pokojowej 20±3˚C. Na podstawie uzyskanych danych obliczono parametry fazy pasożytniczej: długość żerowania, przyrost masy ciała, wskaźnik efektywności żerowania i wydajność żerowania. Długie żerowanie i duża agresywność samic oraz duża ilość krwi pobieranej od żywiciela stwarzają warunki sprzyjające skutecznej transmisji patogenów do żywiciela podczas żerowania.

Wnioski. Badania nad przebiegiem żerowania kleszczy i czynnikami wpływającymi na fazę pasożytniczą mają duże znaczenie w zrozumieniu mechanizmu transmisji patogenów, co może przyczyniać się do opracowania skutecznych metod ograniczania skutków ich działania.

Słowa kluczowe: *Ixodes ricinus*, żerowanie kleszcza, transmisja patogenu.

The process of *Ixodes ricinus* (Ixodida: Ixodidae) feeding and its relation to pathogen transmission

Abstract

Introduction. In Europe the species of ticks most frequently parasitizing humans is *Ixodes ricinus* (castor bean tick, sheep tick). The greatest medical significance of this tick is related to its being a vector for bacteria *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum*, spotted-fever rickettsias, tick-borne encephalitis virus and protozoa Babesia spp., transmitted to the host usually during feeding.

Aim. The aim of the study was to describe the feeding process of the castor bean tick – *Ixodes ricinus* and to analyze the factors affecting this species' capacity to transmit pathogens.

Material and methods. The study investigated the feeding of *Ixodes ricinus* adult forms on rabbits at room temperature 20±3˚C. The following parameters of the parasitic stage were determined: feeding period, increase of female body mass, feeding efficiency index, and tick yield. Long feeding periods and high aggressiveness of females, as well as large quantities of blood drawn from the host, create favourable conditions for effective transmission of pathogens to the host during feeding.

Conclusions. Research on the tick feeding process and factors affecting the parasitic stage is of great importance for the understanding of pathogen transmission, which may contribute to development of effective methods reducing its harmful consequences.

Key words: *Ixodes ricinus*, tick feeding, transmission of pathogen.

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INTRODUCTION

Ticks are worldwide parasites of land vertebrates. Over 22 of tick species frequently attack humans, but also many other species. If their proper hosts are unavailable, they may sporadically feed on human blood [1,2]. In Europe the species most frequently parasitizing on humans is *Ixodes ricinus* (castor bean tick, sheep tick). The components of its saliva may cause skin lesions and allergic skin reactions, but also systemic reactions, including anaphylactic shock [3-5]. However, the greatest medical significance of this tick is related to its being a vector for bacteria *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum*, spotted-fever rickettsias, tick-borne encephalitis virus and protozoa *Babesia* spp., transmitted to the host usually during feeding.

AIM

The paper describes the feeding process of the castor bean tick – *I. ricinus* and analyzes the factors affecting this species' capacity to transmit pathogens.

MATERIAL AND METHODS

Ixodes ricinus ticks were collected with the flagging method in the forest biotope in the vicinity of Lublin. The method consists in dragging a white cloth through vegetation, retrieving the fixed ticks every several minutes and placing them in containers. The species and developmental stage of the collected ticks were determined with the use of a stereoscopic microscope. Female and male specimen used in the experiments were transferred to retaining chambers and kept in the conditions favourable for these developmental stages, i.e. at the temperature 25°C and 75% humidity, determined in earlier studies (Buczek, unpublished).

The feeding process of *I. ricinus* females at 20±3°C was observed on rabbits (*Oryctolagus cuniculus*) that had not previously been infested with ticks. Before feeding, each tick specimen was weighted on an analytical balance exact to 0.0001g. The experiments on the feeding process were inspected daily at the same time, which made it possible to observe changes in the body size of females as they were filling with blood and to collect them immediately after they detached from the rabbit skin. All specimens were weighed again after feeding. On the basis of the obtained data the parameters of the parasitic stage were calculated: feeding period (FP), female engorgement weight (FEW), increase of female body mass (IFBM), feeding efficiency index (FEI) and tick yield (TY). The increase of female body mass represents the change in female body mass as a multiple of the initial body mass. The feeding efficiency index shows the average increase of body mass (in g) over 24 hours; it represents the relation between the feeding time of a female and the body mass attained in this period. The tick yield represents the percentage of females completing their feeding with the weight over 0.1g.

RESULTS AND DISCUSSION

I. ricinus females fed on rabbits for 8-10 days at 20±3°C attaining the average body mass of 0.331±0.033g (Table 1). The average increase of body mass in engorged females during feeding was 236.214±23.665. The examined *I. ricinus* females showed differences in the feeding efficiency index, which varied from 0.027 to 0.050. All females of this tick species made attempts at feeding.

TABLE 1. Parameters of parasitic stage for *Ixodes ricinus*.

Parameter	Min	Max	M	SD
Feeding period FP – (days)	8	10	8.500	0.682
Female engorgement weight FEW – (g)	0.262	0.397	0.331	0.033
Increase of female body mass – IFBM	187.143	283.571	236.214	23.655
Feeding efficiency index – FEI	0.027	0.050	0.039	0.006
Tick yield TY – (%)	100			

The wide host spectrum of *I. ricinus* females, including numerous wild and domestic animal species [6] as well as humans, and tick high aggressiveness, expressed by 100% feeding success, make them a very effective vector of anthropozoonoses. Pathogen transmission is favoured by long feeding time of *I. ricinus* females, sufficient for pathogens to multiply to a number that guarantees the host infection. Research showed that the number of spirochetes 72 hours after *Ixodes scapularis* nymphs had started feeding, increased 300 times [7] with a simultaneous decrease in the expression of OSP A *Borrelia* surface protein, binding bacteria to the intestinal wall of the arthropod, and an increase in OSP C protein, playing an important role in infections [8]. Because infection of the host depends on the number of spirochetes and the concentration of OSP protein, it is most probable to occur 48 hours after the beginning of feeding. However, other studies indicating the possibility of accumulating spirochetes in the cement plug [9] and the capacity to infect the host by some genospecies in a much shorter time [10], suggest that infection may occur at a different time of attachment of ticks to the host's skin or even after their removal (the cement substance remains in the skin).

A long feeding time is particularly important for pathogens (e.g. *Borrelia* spirochetes) multiplying in the intestinal epithelial cells and spreading in the arthropod organs with lymph only after feeding has begun. The pathogens are carried with lymph to the salivary gland cells, and next, with their excretion, to the host. The surface of the *I. ricinus* body surface changes during feeding, visibly growing before it ends. Quick changes related to removal of excess water contained in food and to digestion of its components increase the possibility of the movement of pathogens within the tick body.

Pathogens excreted with tick saliva are transported via salivary glands due to transcytosis, which consists in pathogens entering the cell by endocytosis and leaving it by exocytosis [11]. Infection of the host is favoured by the mechanism of feeding. Tearing of the skin and introducing the tick hypostome as a gutter for the pathogen-containing

saliva partly bypasses the immunological barrier of the skin. The study by Crippa et al [12] showed that animals do not become infected if spirochetes *Borrelia burgdorferi sensu stricto* and *Borrelia afzelii* in the homogenate of *I. ricinus* nymph tissues are placed on the skin; however, if pathogens are injected into it, infection occurs.

Pathogen transmission is favoured by the manner of taking food consisting of blood components and other dissolved tissues of the host. It consists in alternate injecting of saliva and absorbing of food [13]. Taking large quantities of food, composed of proteins and lipids, by *I. ricinus* females creates an environment conducive to pathogen development.

Because digestion in ticks is intracellular, microorganisms present in the intestinal crypts are not directly exposed to proteolytic enzymes. The action of those enzymes changes along with the feeding stage, increasing between 48th and 72nd hour from attachment to the host's skin, which may lead to the death of pathogens transported inside the cells in the process of endocytosis. After that period, however, the enzyme activity decreases, which creates favourable conditions for the development of microorganisms transported to the intestinal lumen during the final stage of feeding [14]. The observed differences in feeding efficiency among *I. ricinus* females may affect their capacity to transmit pathogens. This issue, however, requires further investigation.

The components of salivary glands excretion indirectly influence pathogen transmission by a deep, pharmacological modification of the skin at tick-feeding sites [15-20]. The cement plug and liquid saliva produced by *Rhipicephalus appendiculatus* contain antibacterial substances acting similarly to lysozyme. It is supposed that they play an important role in transmission of lysozyme-resistant pathogens, such as rickettsias, tick-borne encephalitis virus, and *Borrelia spirochetes* [21]. Antibacterial substances in saliva and cement plug probably have a protective function against destructive physiological microflora of the host's skin. Anti-inflammatory, lytic, anticoagulant and immunosuppressive activity of the components of tick saliva, fundamentally important for the physiology of those blood-feeding arthropods, naturally facilitates transmission of pathogenic microorganisms and accelerates spreading from the site of penetration into the host's tissues.

Research on the tick feeding process and factors affecting the parasitic stage is of great importance for the understanding of pathogen transmission, which may contribute to development of effective methods reducing its harmful consequences.

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