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PRELIMINARY STUDIES ON NATURAL ENEMIES OF HARD TICKS AND METHODS OF THEIR POPULATION CONTROL IN LATVIA

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Abstract: Hard ticks Ixodidae (Acari) transmit pathogens causing dangerous diseases such as tick-borne encephalitis and Lyme disease. These tick species in Latvia are dog tick *Ixodes ricinus* and taiga tick *Ixodes persulcatus*. The use of acaricides to control tick populations raises concerns over environmental pollution. Environmentally friendly way to control ticks is necessary to develop. Parasitoid tick wasp *Ixodiphagus hookeri* (Hymenoptera, Encyrtidae) are natural enemies of Ixodidae ticks. The aim of the current research was to study natural enemies of *Ixodes* spp. and determine causes of tick mortality, and development of methods for tick rearing in laboratory conditions. In order to carry out the artificial feeding of ticks, a prototype of a tick feeding device using skin-imitating membrane was created.

Key words: parasitic wasp, parasite, Ixodidae, *Ixodiphagus hookeri*, entomopathogenic fungi

Introduction

Hard ticks *Ixodes ricinus* (Fig. 1) and *Ixodes persulcatus* are found across Europe. Ticks are ectoparasites feeding with animal and human blood. Since hard ticks often carry bacterial or / and viral pathogens, they are vectors for potentially lethal diseases, such as tick-borne encephalitis (TBE) and Lyme disease (Hillyard, 1996).

The number of Lyme disease cases in Latvia has dropped considerably in the last 5 years (from 612 cases in 2017 down to 272 in 2022) Latvian Center of Disease Prevention and Control data (CDPC, 2023). The number of reported tick-borne encephalitis (TBE) cases remain stable at around 200 every year, as shown by the recent data collected by the CDPC. According to the WHO (2020), TBE is the most prevalent arthropod-borne disease in Europe.

Vaccination against tick-borne encephalitis is an effective tool for disease control, still more measures are needed to actively control tick populations. Synthetic acaricides can be used as a short-term solution, there are valid concerns over long-term pollution caused by man-made "forever-chemicals" as well as growing resistance towards commonly used acaricides. A recent study showed that ticks over time become resistant towards common man-made acaricides (Obaid et al., 2022).

Figure 1. Female of dog tick *Ixodes ricinus* (Photo: I. Salmane)

Use of plant-derived alternatives (natural oils), vaccines and introduction of tick control agents, pathogens (fungi, bacteria, viruses) and parasitoids are essential (Jamil et al., 2022; Obaid et al., 2022). Studies have found at least 58 pathogenic fungal species that kill Astigmata, Oribatida, Prostigmata, Mesostigmata and Ixodida mites (Chandler et al., 2000; Ravensberg, 2010). *Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa*, *I. fumosorosea* and *Lecanicillium lecanii* infect Ixodida ticks under natural conditions (Perinotto et al., 2012). Many mycosecticides have been developed with the fungi *B. bassiana* and *M. anisopliae* (Copping, 2009; Fernandes et al., 2012).

At the beginning of the 20th century, endoparasitic wasp of the genus *Ixodiphagus* (Hymenoptera, Encyrtidae) was discovered to parasitize *Ixodes spp.* ticks (Hu et al., 1998; Bohacsova et al., 2016; Sormunen et al., 2019; Gaye et al., 2020). *Ixodiphagus* wasps lay eggs in tick larvae and nymphs, devour their internal organs during the larval development and then pierce the back of a tick with their mouthparts. Female wasps lay significantly fewer eggs in blood-sucking larvae than in nymphs. Eggs develop only in nymphal ticks which are fed by blood. (Hu et al., 1998; Takasu & Nakamura, 2008). An adult wasps emerge from the tick about 20–57 days after the tick falls off the host (Hu et al., 1998; Bohacsova et al., 2016). One female *Ixodiphagus hookeri* can parasitize two blood-sucking tick nymphs (42–50 eggs in each nymph) or three non-blood-sucking nymphs during her lifetime (Takasu & Nakamura, 2008). In Europe, *I. hookeri* has a wide variety of tick hosts: *Ixodes ricinus*, *I. persulcatus*, *Dermacentor reticulatus*, *D. pictus*, *D. marginatus*, *Haemaphysalis concinna*, and *Rhipicephalus sanguineus* (Buczek et al., 2021). Parasitoid wasp *I. hookeri* (Hymenoptera, Encyrtidae) might offer a new environmentally friendly way of controlling tick populations (Hu et al., 1998). Method has limitations, such as the necessity to constantly reproduce and reintroduce the parasitoids, as the parasitoid coexist naturally with the pest (ticks) and cannot completely eradicate the latter. Feeding

of ticks using artificial membranes is becoming more common, as modern materials make it possible to emulate animal skin, with appropriate thickness and texture.

Institute of Biology, University of Latvia in cooperation with LTD "Kukaiņu garāža" started a study of biological control of hard ticks *Ixodes spp.* in Latvia. The objectives of research were: 1) the study of causes of mortality and natural enemies of *Ixodes spp.* in climatic conditions of Latvia; 2) the development of methods for *Ixodes* tick control using their natural enemies; 3) the development of a prototype for the feeding and reproduction of *Ixodes spp.* ticks as host of parasitic wasps.

Materials and methods

Collection of Ixodes spp. and determination of mortality causes of ticks

During the 2021 season ticks of the genus *Ixodes* were collected at Ziemeļblāzma (Skulte parish) by the flag dragging method. Fed ticks were collected from migratory birds in Pape ornithological station (Latvia) and from cats in Salaspils and Ziemeļblāzma. The presence of parasitoids and pathogens was observed immediately after collection. Repeated inspection of tick individuals was carried out after storage in an incubator at +23 ± 2 °C temperature for 60 days. The mortality factor was referred to one of the categories: parasitoids, bacteria, nematodes, pathogenic fungi, mechanical or another cause. Specimens with symptoms of infection (cuticle covered with fungal mycelia or conidia) were used for entomopathogenic fungi isolation.

Isolation and determination of entomopathogenic fungi

Collected dead ticks were surface sterilized in 1% sodium hypochlorite for 30 sec., rinsed three times in sterile distilled water and placed in humid conditions to stimulate fungal growth and sporulation. Preliminary identification of fungi was confirmed by slide preparation. For specimens with conidial cushions preparations of conidia were obtained by film method. The shape and size of conidia were examined using a microscope fitted with a micrometer scale. Squash preparations of various infected tissues were viewed in light microscope Olympus CX41 with magnification of 400× (Lacey & Brooks, 1997). Agar-coated slide technique and staining with Lactophenol Cotton Blue were used for observation of sporulating structures and spores. Fungi were isolated and maintained on Malt extract agar. Keys for the identification were used (Lacey & Brooks, 1997, Samson et al., 1988).

Tick storage

Collected alive *Ixodes ricinus* ticks were transferred to vials with nylon fabric end caps (Fig. 2 A) and stored in jars halfway filled with wet sand for transfer to the laboratory. Afterwords vials were put into desiccators with saturated magnesium sulfate solution to ensure 98% humidity. The desiccators were placed into incubators set to +22 °C with a 16 : 8 day–night light cycle for long-term storage.

Feeding unit construction

The feeding units were built according to Oliver et al. (2016) with some minor alterations. Polycarbonate pipe (diameter 14 mm) was cut into equal pieces (6 cm in length). Two-component silicone rubber (Smooth-On with Shore hardness of 00–10 and 00–50 for nymphs and adult ticks, respectively) was mixed with petroleum ether (PE 40 °C) in the ratio of 1 : 2 (by volume). Thin pieces of rayon paper (12 g/m^2) were impregnated with the silicone-PE mixture, which was then dispersed using a soft silicone wiper until no more silicone collected on the brush. The sheets were left to air dry for 24 hours. After drying, the silicone paper was hand-pressed to improve tackiness. Greenhouse silicone (A-10 Shore hardness, with no added preservatives) was applied to the edge of the tubes, which were then attached to the silicone paper and slightly twisted to ensure a complete seal. The feeding units were left to dry for another 24 hours. Then a scalpel was used to detach the feeding units from the sheet by carefully cutting around the tube. The membrane was tested for leaks using 70% ethanol for 10 minutes. Faulty units were discarded. The assembled membranes had a thickness of 14–20 µm for 00–10 silicone and 18–28 µm for 00–50 silicone, respectively, which corresponds well to the results obtained by Oliver et al. (2016).

Preparation of tick attracting extract

The hair extract was prepared according to Krull et al. (2017). Dog hair was collected from the dog that at least one year was not treated with tick/flea repellents. 10 g of hair was cut into small pieces and suspended in 50 mL of dichloromethane (DCM, analytical grade). The mixture was stirred with mild heating (+40 °C) for 20 minutes. DCM was separated and a new portion of the solvent (25 ml) was added and stirred as before. Extraction was repeated two more times. All organic extracts were combined and evaporated down to 7 mg of dog hair per 1 ml of solvent. The extract was stored in the freezer (–20 °C) and used within the same week.

Tick feeding

Tick feeding was attempted using the procedure developed by Oliver et al. (2016) with alterations. 160 µl of dog hair extract was poured into the feeding unit and left to dry for at least 2 hours. 4,5 ml of thawed out bovine blood was used for each feeding unit. Each blood portion was poured into feeding plate (or beaker) and supplemented with 15 µl of aqueous glucose solution (1 g/l), 45 µl of the 100x antibiotic-antimycotic solution (BioReagent, 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml), 4 µl of aqueous 0,1 M ATP (disodium salt) solution. The blood meal was warmed up to +37 °C. Twelve adult ticks were placed into the feeding units (Fig. 2 B), which were inserted into the feeding plate in direct contact with the blood. The top of the feeding unit was covered with Parafilm. The assembled units were placed in a water bath set to +37 °C. The blood was changed every 12 hours, and the feeding units were carefully rinsed with sterile buffered saline to remove old blood prior to adding new blood.

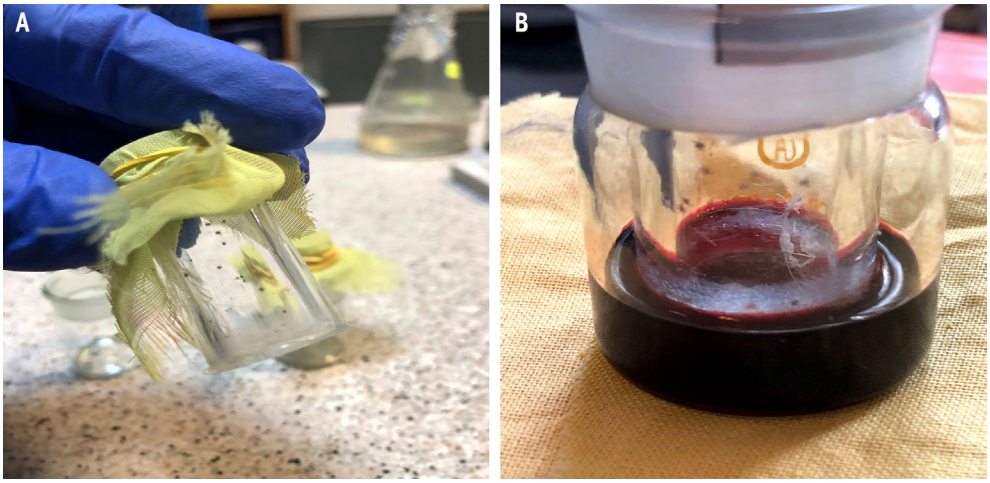

Figure 2. A – tick storage vials and B – tick feeding unit

Results and discussion

The examination of 89 fed ticks from animals was performed immediately after collection. The presence of parasitoids and pathogens was not observed immediately after collection. After storage in a desiccator at +22...+25 °C for 60 days, a repeated inspection of tick individuals was carried out. It was established that 16 ticks or 18%, were dead. In two cases (2%), a hole in the tick's abdomen was found, indicating hatching of a parasitic wasp. The wasps themselves were withered and it was not possible to determine the species.

500 ticks collected by the flag dragging method were examined after 60 days, as according to the literature, parasitic wasps develop for 30–70 days (Collatz et al., 2011). In the nymph stage were 263 ticks and 237 were imagoes. Males were 1.6 times more in number than females. It was concluded that after 50–60 days 122 ticks or 24% were dead. Causes of mortality for both groups of ticks are shown in Fig 3. A hole in the abdomen of three ticks was observed, indicating hatching of parasitic wasps. In several cases, ticks drowned in droplets of condensate (mechanical cause of death). In other cases, the cause of death has not been determined.

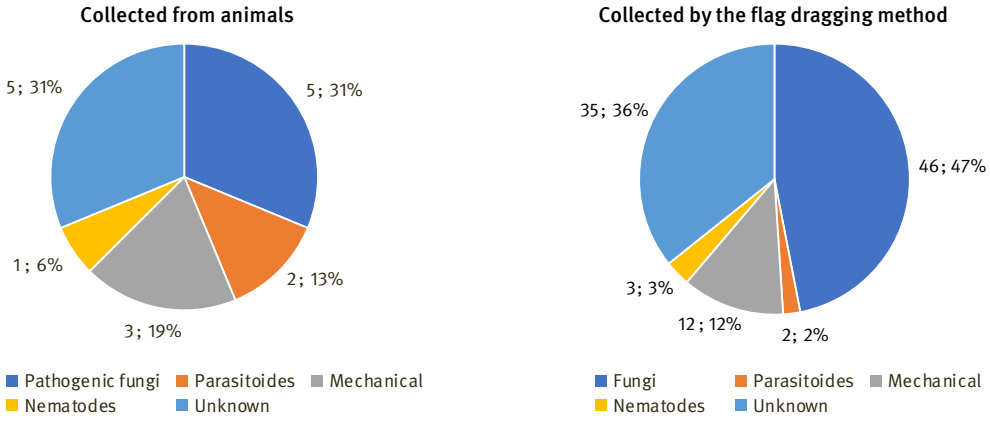

Figure 3. Mortality causes of ticks that died after 60-day incubation. In total dead 16 ticks collected from animals and 122 collected by flag dragging method were investigated

Infection with entomopathogenic fungi was found in 5.6% of all ticks collected from animals and 9.2% of all ticks collected by the flag dragging method. 36 isolates were isolated in pure cultures. Among them *Beauveria bassiana* (8 isolates), *Lecanicillium lecanii* (6 isolates), *Metarhizium anisopliae* (4 isolates), *Beauveria sp.* (6 isolates) and 10 isolates were not identified. Our study showed that tick parasitoid wasps are also found in Latvia, as 2% of the ticks collected from animals and 0.6% of the tested ticks collected by the flag dragging method had a hole in abdomen.

Tick survivability

High humidity conditions eventually result in a rapid fungal growth within the storage unit and causes death of ticks, making it difficult to preserve ticks in laboratory till next summer. High mortality during feeding for both nymphs and adult ticks after 2 months of uninterrupted storage over saturated magnesium sulfate solution was observed. It is possible that repellent residue used in previous season from collected dog hair used in preparation of attracting extract, might negatively affect ticks in the feeding chamber. Shampoo residue from occasional grooming also might be a source of potentially harmful chemicals. Collecting hair samples only from farm animals (sheep, goat and cow hair) with no history of acaricide/repellent use might be a viable solution. Moreover, tick excrements, a phagostimulants used by Oliver et al. (2016) was not available during tick feeding experiments. Additional use of tick excrements as attractor may improve the feeding process.

In the future to improve the membrane feeding prototype, different phagostimulants should be used (or a combination), such as tick excrements, extract of tick excrements as well as hair collected from wild or free-range animals. Different temperature regimes should be tested as well (for example, room temperature during tick feeding).

Conclusions

Populations of the *Ixodes* ticks in Latvia are not regulated by parasitoids (level of mortality < 2.3%).

The impact of pathogens on hard ticks was low – mortality rate of all inspected ticks was 5.6–9.2%. 36 isolates of pathogenic fungi, associated with *Ixodes spp.,* were isolated. Isolates of determined pathogenic fungi were representatives of genera *Beauveria, Metarhizium* and *Lecanicillium*. As next step, it is necessary to carry out molecular identification of fungal isolates. In future it is necessary to improve tick feeding technics in laboratory conditions.

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