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Editorial

ENTOMO-PATHOGENIC NEMATODES: A POTENTIAL TOOL FOR BIOLOGICAL CONTROL OF TICK(S)

Tick infestations cause huge economic losses to the dairy industry both directly (reduced weight gain and milk production, damage to hides and skins, anaemia, and even mortalities) and indirectly by transmitting various disease-causing pathogens such as *Babesia* spp. and *Anaplasma* spp. (Ghosh *et al.* 2007). Presently, the application of chemical acaricides remains the primary method of tick control worldwide. However, the indiscriminate and extended use of these chemicals often produce undesirable effects, such as contamination of milk and meat products with insecticide residues, environmental pollution and development of tick resistance to the insecticides which leads to use higher concentration of insecticides. Therefore, alternatives such as breeding of resistant animals, tick vaccines, pheromone-assisted control, botanical acaricides and biological control by pathogens, predators and parasitoids have been explored with a view to reduce dependence on the use of chemical acaricides.Biological agents such as ox-peckers, fungi and parasitic wasps are known to control ticks efficiently but no such agents are used commercially. Practical biological control of insect plant pests is well established alternatives to chemical pesticides. For control of arthropod pests, one notable strategy is to develop entomopathogenic nematodes as agents to control them (Samish and Glazer2001).

Entomopathogenic nematodes

Nematodes are organisms grouped in the phylum Nematoda and are among the most abundant groups of invertebrates on the surface layers of the earth, rivalling the Arthropoda in biodiversity and species abundance. While most nematodes are free-living, numerous can be associated with invertebrates such as insects, mites and molluscs, ranging from casual to obligate parasitism and pathogenesis. Among these, the entomopathogenic nematodes (EPNs) of families, Steinernematidae (Travassos) and Heterorhabditidae (Poinar) have been used successfully in various locations around the world to control different insect pests (Grewal *et al.* 2001). EPNs in the families Steinernematidae and Heterorhabditidae (Enlisted in Table 1) are those which vector insect–pathogenic *Xenorhabdus* and *Photorhabdus* bacteria, respectively (Gaugler and Kaya 1990). Both *Xenorhabdus* spp. and *Photorhabdus* spp. belong to the family Enterobacteriaceae are gram-negative, motile, facultative anaerobic rods and non-spore forming. However, this number is increasing as the number of novel species being described is growing every year.

Entomopathogenic nematodes have been recovered worldwide and are likely to be globally distributed and are essentially ubiquitous. EPNs are among the beneficial bio-control agents that are frequently used for pests control in agriculture, forestry and health (Grewal *et al.* 2005). As bio-control agents, they possess the advantages of having a broad host range and no known negative effect on both environment and non-targeted organisms. They can search and kill their hosts rapidly, are easily massed produced *in vivo* and *in vitro*, are susceptible to genetic selection of desirable traits and, are easily applied using conventional equipment. In addition, they can be used with many chemical or biological pesticides or adjuvants (Reis-Menini *et al.* 2008). In contrast, their disadvantages are that their broad host range can possibly include beneficial insects, are poorly tolerant to environmental conditions such as soil moisture content, UV radiation and have limited shelf-life.

Life cycle of entomopathogenic nematodes

The life cycle of both *Steinernema* and *Heterorhabditis* species comprises non-feeding, free-living infective juvenile (IJ) that infects the insect host in the soil environment and develop into the adult life stage as shown in Figure 1. The IJ stage is a form resulting from the depletion of food resources and adverse environmental conditions and only one that can occur outside of an insect. The IJ stage infects the host; then develops to J4 and G1 (adult 1st generation). Then produce eggs (after mating) that will develop to J1. In abundance of food, J1 will successively molt to J2, J3, J4 and G2 (2ndgeneration adult). This process will repeat until G3 (3rd generation) depending on the availability of food. When food is limited, J1 will molt to J2, pre-I and IJ3 that will emerge from the cadaver to search for a new insect host (Brivio and Mastore 2018).In addition, steinernematids reproduce amphemictically, whereas heterorhabditids can reproduce

Table 1. Classification of EPNs.

Family	Steinernematidae	Heterorhabditidae	Reference
Genus	1. Neosteinernema: comprises only one species, *Neosteinernema longicurvicauda* Nguyen & Smart (Rhabditida: Steinernematidae). 2. *Steinernema* (type genus) with around100 recognized species	Contains only one genus, viz, *Heterorhabditis*, with 20 species.	Malan and Ferreira 2017

either hermaphroditically or amphemictically. The IJs of nematodes is generally used in pest control. When the juveniles encounter a susceptible host, they penetrate the hemocoel by using enzymes and mechanical force. Once inside the host, the nematodes release symbiotic bacteria (*Xenorhabdus* spp. or *Photorhabdus* spp.)that colonize and kill the host.

Nematode–tick interaction

Nematodes are known to enter the body of insects mainly via natural orifices. Nematodes virulent to engorged *Amblyomma americanum* females were attracted towards the natural apertures of the ticks or trying to penetrate between *Rhipicephalus (Boophilus) annulatus* mouth parts. Ticks can be killed by the injection of a single nematode but axenic nematodes (i.e. those without symbiont bacteria) are unable to kill ticks even though they are pathogenic to insects. This demonstrates the essential part is played by the symbiotic bacteria of nematodes in killing the ticks. A few days after juvenile nematodes penetrate or are injected into ticks, all or most of them die inside their tick host. Although in rare cases they have survived as IJs or even started to develop within the tick, they have not completed their life cycle. However, when the cuticle of ticks was slit artificially before their infection, the nematodes were able to complete their life cycle. Although steinernematids and heterorhabditids infect the ticks in a similar pattern, the steinernematids appear to prefer to search for hosts at or near the soil surface, characterized as ambushers, while the heterorhabditids are adapted to search deeper in the soil and are defined as cruisers (Grewal *et al.* 2005).

Nematode virulence to ticks

The susceptibility of ticks to infection by EPNs can vary according to the tick species, stage of tick development, EPN strain and exposure time of the ticks to EPNs (Samish *et al.* 2008). Studies under laboratory conditions have demonstrated that EPNs represent a promising alternative to develop control strategies for *R.* (*B.*) *microplus* (Samish *et al.* 2008, Monteiro *et al.* 2012, Singh *et al.* 2018). Also, application of different EPNs is shown to be effective against *Amblyomma americanum* (Kocan*et al.* 1998), *R.* (*B.*) *annulatus* (Alekseev *et al.* 2006), *Ixodes ricinus* (Hartelt *et al.* 2008) and *Dermacentor nitens* (Monteiro *et al.* 2014). The use of EPNs against the non-parasitic phase of engorged female ticks can be effective, as these engorged females seek environments with high moisture and protected from solar radiation at the time of oviposition, a condition that also favours the survival of EPNs (Grewal *et al.* 2001).

Till date several heterorhabditids and steinernematid strains have been tested for their antitick activity and shown varying degrees of virulence. Heterorhabditidsare generally more virulent to ticks than steinernematid nematodes (Singh *et al.* 2018, Goolsby *et al.* 2018). In most cases, strains virulent to one tick species and one stage were also highly virulent to other tick species and stages (Samish and Glazer 1991). Fully engorged argasid and ixodidfemale ticks were generally the most sensitive to EPNs; unfed adult ticks were less sensitive and immature stages the least sensitive, while the eggs were fully resistant (Samish and Glazer 1991). Females seem to be more sensitive when ovipositing than in their pre-oviposition stage.

The EPN infections do not interfere with the metabolic conversion process or production of the necessary nutrients for production of eggs during the pre-oviposition period in nematode exposed engorged female ticks leading to non-significant effect on the pre-oviposition period (Vasconcelos *et al.* 2004, Singh *et al.* 2018). Whereas, the infection by EPN IJs interferes with the oviposition of treated engorged female ticks leading to significant reduction in egg mass weight of treated ticks. Also, different strains or species of IJs may have different influences on the fecundity of engorged females of different tick species and is more pronounced for *Heterorhabditis* strains (Carvalho *et al.* 2010). Further, EPN exposure also lead to reduced hatchability in tick eggs and may be due its possible deleterious action on the oviposition and/or embryonation process, by interfering in the steps of forming oocytes, fertilization,

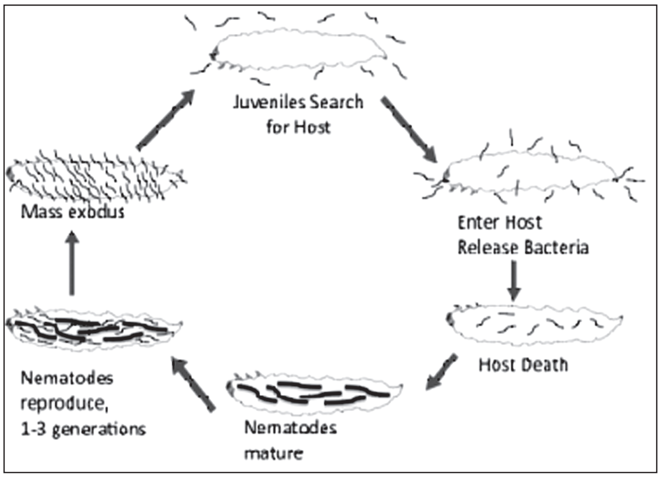

Fig 1. Life cycle of entomopathogenic nematode.

water absorption by the cuticle and waxing of the eggs by Gene's organ (Silva *et al.* 2012). Reports indicate that longer exposure times allow a greater number of nematodes to locate and penetrate the host, which apparently increases the effects on the biological parameters of engorged female ticks (Singh *et al.* 2018). According to Hill (1998), exposure of ticks to nematodes for a short period reduces the number of IJs that successfully penetrate engorged females, thus decreasing the lethality of the infection.

While feeding on a host, ticks are generally resistant to nematodes except on very moist feeding sites. In a recent study, two EPN species *Steinernema riobrave* and *Heterorhabditis floridensis* were tested for *in-vivo* efficacy against southern cattle fever tick, *R. (B.) microplus*. Twelve heifer calves were experimentally infested three times at 1-week intervals with 125 mg of larvae of southern cattle fever ticks on days 0, 7, and 14. On Day 21, the animals were randomly divided into three treatment groups and sprayed with 5,000 IJs/ml. *S. riobrave* and *H. floridensis* affected all life stages of the tick, and caused 14.5 and 25.4% reduction in production of adult engorged female ticks during the first 21 days post treatment. The nematodes significantly affected the reproductive parameters of treated ticks, but not egg hatchability (Goolsby *et al.* 2018).

Reis-Menini *et al.* (2008) reported EPNs particularly *S. glaseri*are compatible with chemical acaricides and can be used together with an organophosphate pesticide for control of ticks in an integrated approach.Recently, efficacy of infective juveniles of several heterorhabditids and steinernematid strains were reported against engorged adult females of the multi-acaricide resistant strain of *R. (B.) microplus* (Singh *et al.* 2019). Thus, EPNs might be used as part of an integrated approach to control tick resistant to various classes of acaricides. The EPNs do not undergo their normal propagative cycle within ticks (Zhioua *et al.* 1995), thereby supporting the hypothesis that ticks are poorer nematode hosts than insects. Nematodes are generally isolated from nature by using insects (larvae of the wax moth *Galleria mellonella*) as bait however, in future trials, if ticks are used as bait to attract EPNs in nature, it may be expected that far superior types of nematodes could be isolated for tick control.

The wide genetic diversity of nematode strains suggests that antitick virulence could be further increased by screening, selection and/or genetic manipulation. The preferred ecological habitats of nematodes and off-host tick stages are often similar thus increasing its potential for the control of tick stages hiding in the animal sheds. The biological control of a pest is often based on an arsenal of biocontrol agents that target different species and stages, are more efficient in specific ecological habitats or particular seasons, etc., and can replace some traditional chemical pesticides. Therefore, the inclusion of EPNs in an integrated tick-control program can be of immense help and should be seriously considered.

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