Review Article

Expanding Biological Control Spectra of Entomopathogenic Nematodes to Tick Pests

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Abstract | The entomopathogenic nematode (EPN) products are developing to control crop pests. Yet, their commercialization is limited to few niche markets. Meanwhile, mounting concern over chemical acaricides - because of environmental pollution, human safety, and developing tick resistance to acaricides has promoted EPNs as their replacements. Such replacements will widen EPN uptake and further stimulate the organic product market. Safe/effective management via EPNs has become more evident as ticks developed resistance to nearly all classes of acaricides. No insects have developed resistance to insecticidal nematodes. Although EPNs do not use their natural reproductive cycle within tick cadavers, their usage bodes well to control key tick species. Their applications will avoid not only ticks-direct damages but also many tick-borne diseases. These latter can even inflict humans which constitute a substantial public health concern worldwide. Fortunately, the basics/experience assembled in using EPNs against other arthropods can assist in developing tick biocontrol methods. As EPNs naturally live in/on the soil, where 95% of tick populations infesting livestock are found, EPNs are favorable biocontrol agents of ticks in infested grasslands/pastures. Moreover, sophisticated techniques can optimize their efficacy against ticks on the infected animal skins. They use "smart sprayer" to remotely manage ticks on animals, a sprayable gel to protect the nematodes on cowhides from ultraviolet radiation/desiccation, or applying proper oil emulsion of EPNs to prolong their efficacy. Other discussed features to improve EPN efficacy against ticks engage EPN delivery/persistence, priming them/their bioactive materials, and introducing additional devices to reform their biocontrol potential.

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Introduction

Pest management is at a crossroads with worldwide changes becoming continuously more apparent. Due to currently mounting conflict, mass migration, and various plagues, many peoples are threatened by a lack of access to the resources needed to avoid localized famine and increased poverty in many parts of the world. Meanwhile, losses in food production solely induced by insect pests were reported to reach 45% annually (Battu *et al*., 2023). Numerous issues challenge sound exploitation for positive trends of food production-insect pest interactions. Hence, a full beneficial spectrum of these interactions should address biopesticidal activity against main pests of our animal wealth. In this vein, ticks and tick-borne diseases (TBDs) inflict a substantial public health concern in both developing and developed countries. For example, sixteen of the eighteen TBDs are known to infect humans in USA (Iowa State University, 2023). These TBDs are frequently diffused by the bite of ticks but may also be diffused by blood transfusion. Big economic losses regarding production of meat and milk of livestock due to ticks and TBDs are being recorded. Cruz *et al.* (2016) reported huge losses in production of hundred thousand of cows in tropical and subtropical regions due to exposures to infestations by ticks and TBDs. Such losses are exemplified annually by the southern cattle fever tick, *Rhipicephalus microplus* Canestrini in wide areas there. Furthermore, ticks and TBD inflict global damages of billions of dollars for farmers annually (El-Alfy *et al*., 2022). In other regions, many countries like Egypt are in dire need of these animal products in order to save hard currency regarding their importation (El-Ashram *et al*., 2019).

In this vein, entomopathogenic nematodes (EPNs) possess virtually all the attributes of an ideal biological control agent, BCA (Tarasco *et al*., 2023). Oppositely, negative aspects of chemical pesticides used against herbivorous pests mainly comprise health hazards, environmental pollution, and development of pest resistance. These negatives have created global markets for EPNs. However, EPN usage on crop pests is still limited to relatively few niche markets due to unaffordability, occasional low efficacy, and lack of public awareness for their ecological and sustainable gains (Askary *et al*., 2017). On the other, information is scanty about EPN commercial products for controlling pests of livestock. Currently, controlling most of the related tick species is mainly dependent on acaricidal applications. Unfortunately, the excessive usage of chemical acaricides has been developing negative consequences; increasing evidence of developed tick resistance to acaricides and mounting environmental pollution (Singh *et al*., 2018). The Food and Agriculture Organization of the United Nations, FAO (2024) confirmed current increase of such acquired resistance by ticks due to malpractices in the acaricidal application, using substandard products, and improper strategies to retard the emergence of tick resistance. Because acquired resistance against nearly all classes of acaricides involving macrolactones has been proved, other anti-parasitic chemicals have been tested against ticks parasitizing livestock. Nonetheless, El-Ashram *et al.* (2019) documented a para-acaricide (ivermectin) resistance among tick populations in Egypt. Moreover, the cost of such chemicals, especially for most farmers in developing countries, is often prohibitive. Thus, new strategies for managing such pests are direly needed. Furthermore, the one-host tick such as *Boophilus decoloratus* Koch (Acarina: Ixodidae) can usually manifest acaricide resistance quicker than the three-host tick species like *Amblyomma variegatum* Fabricius and *Rhipicephalus appendiculatus* Neumann (Acarina: Ixodidae) (Kaaya *et al*., 2000). In contrast, expanding the usage of BCAs such as EPNs to control pests of domestic animals will help to further stimulate the organic food market in both livestock and plant production.

The present study focuses on extending useful biocontrol spectra of EPNs to tick pests of domestic animals. It considers the importance of these EPNs especially from the standpoint of managing pests of livestock. The study briefly presents the basic ecology and biology of both EPNs and their tick hosts as a rationale for astute applications of their related biocontrol activity.

Initially, EPN processing in terms of their sampling, extraction, counting, and identification techniques is highlighted to refine the current common techniques. As their biocontrol strategies are targeted chiefly at pests of plants, EPNs-biocontrol potential should be expanded to safe and effective control of additional key pests like ticks. Due to their aforementioned public health concern and economic importance, ticks infesting domestic animals are highlighted as EPN hosts. Meanwhile, this review discusses emerging and novel techniques to optimize related EPN effectiveness on ticks infesting livestock. The techniques include delivery/persistence, priming them/their bioactive insecticidal materials, and introducing advanced approaches to reform their biocontrol activity.

A brief on the biology/ecology of the nematodes and their tick hosts

Species of the two genera *Heterorhabditis* and *Steinernema* in the families Heterorhabditidae and Steinernematidae, along with their symbiotic bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively are the main domain of EPNs (Koppenhöfer *et al.*, 2020). They are naturally found in/on soil within a variety of habitats like farmed fields, forests, deserts, grasslands, and coastal beaches (Pant *et al.*, 2024). Their presence covers all continents excluding Antarctica. A full biocontrol spectrum of EPNs has already addressed activities of both the symbiotic bacterial species individually and nematode-bacterium complex in current and emerging cropping systems (Abd-Elgawad *et al.*, 2017; Tarasco *et al.*, 2023). The nematode-bacterium complex, known herein as EPNs, can continuously reproduce in most of their host populations which bodes well for their exploitation in prolonged suppression of general arthropod pests under favorable conditions.

The only free living EPN stage, a specialized third-stage juvenile (J_3), is the infective juvenile (IJ) or called the dauer juvenile. Its function is to search for and enter into the body of its arthropod host. Its foraging ranks between two processes within the ambusher–cruiser continuum (Askary *et al.*, 2018). These foraging strategies have been defined for some EPN species, but their practices are usually modified by other factors such as volatile cues released from plant roots that are attacked by the insect hosts and/or the host insects themselves. Also, soil characteristics like moisture, texture, and temperature can adjust the foraging strategies (Shapiro-Ilan *et al.*, 2018). Furthermore, EPN application may be restricted to defined environmental conditions because of their sensitivity to low humidity or desiccation as well as high silt/clay fraction and/or manure concentration in soil habitat. In contrast, cues like volatile organic compounds (VOCs) liberated by IJs can negatively impact the behavior of root insect herbivores (Helms *et al.*, 2019). Clearly, these VOCs may back the gains of using EPNs in biocontrol activities. Overall, IJs entry into bodies of their host insects may physically occur via the host's natural openings (mouth, anus, and spiracles) or via boring into the attacked insect at portions with faint cuticle (Gaugler, 2002). On entering the hemocoel by the IJs, the symbionts are released via EPN regurgitation/defecation and begin to function in the host body. These bacteria usually induce insect mortality via septicemia in a short time; 1-3 days (Abd-Elgawad, 2017). For *Steinernema* species, IJs grow within the insect hemocoel into adults (females/males) and reproduce for several cycles depending on the capacity/size of insect body to produce males and females, except for *S. hermaphroditum* (Koppenhöfer *et al.*, 2020). On the opposite, IJs of *Heterorhabditis* spp. develop to hermaphroditic adults in the onset generation. Their following generations typically contain females, hermaphroditic individuals, and males. The reproductive cycles of species in both genera on their common hosts are illustrated elsewhere (Abd-Elgawad, 2017). Usually, thousands of IJs with the symbionts in their digestive system migrate out of the host cadaver, after depletion of their diet source, to forage for other arthropod hosts. The symbionts rely on the nematodes, their partners, to enter into a novel susceptible insect. These bacteria produce antibiotic compounds that can disable secondary insect host infestations and supply proper diet for the EPN feeding while reproducing in the host cadaver. Certain species of nematode-bacteria complexes on their specific hosts were examined to find out the role of the nematodes and their symbiotic bacteria in defeating the physical barriers and immunity system of their insect hosts. Species of *Photorhabdus* (Abd-Elgawad, 2021a) and *Xenorhabdus* (Abd-Elgawad, 2022a) possess sober arsenal of chemicals against insect hosts. Nonetheless, these bacteria presumably have still many of such compounds to be detected. Such a bacterial arsenal is assumed to help supplementary venom proteins provided by the nematodes so that the dyad can cause host mortalities (Lu *et al.*, 2017; Chang *et al.*, 2019; Abd-Elgawad, 2023).

Some reports (Samish *et al.*, 2008; Albogami, 2024) indicated that EPNs do not use their normal reproduction cycle within tick cadavers of specific ticks. In other words, numerous tick species are quite susceptible to infection with consequent lethality caused by various EPN species/strains, but they do not seem to be efficient hosts for the EPN reproduction (Goolsby *et al.*, 2018). Nonetheless, the probit/logit coefficients measuring the response of the tick mortality to variables such as EPN concentration, dose, or tick species-exposure time to the nematodes showed promising results. Such coefficients evidenced significant variable-response relationships in terms of the lethal concentration values, LC_{50} and LC_{90} (El-Roby *et al.*, 2018; Aquino-Bolaños *et al.*, 2019; Albogami, 2024); lethal dose, LD_{50} (Goolsby *et al.*, 2018); and lethal time, LT_{50} (El-Roby *et al.*, 2018; Albogami, 2024). Thus, it is probable that tick lethality induced by EPNs seems to be due to the quick proliferation of the EPNs-mutualistic bacteria within the tick's body, as the EPN-infective juveniles die shortly after entering the insect body (Samish *et al*., 2008; El-Roby *et al*., 2018; Albogami, 2024). Clearly, no insects have developed resistance to EPNs (Abd-Elgawad, 2017).

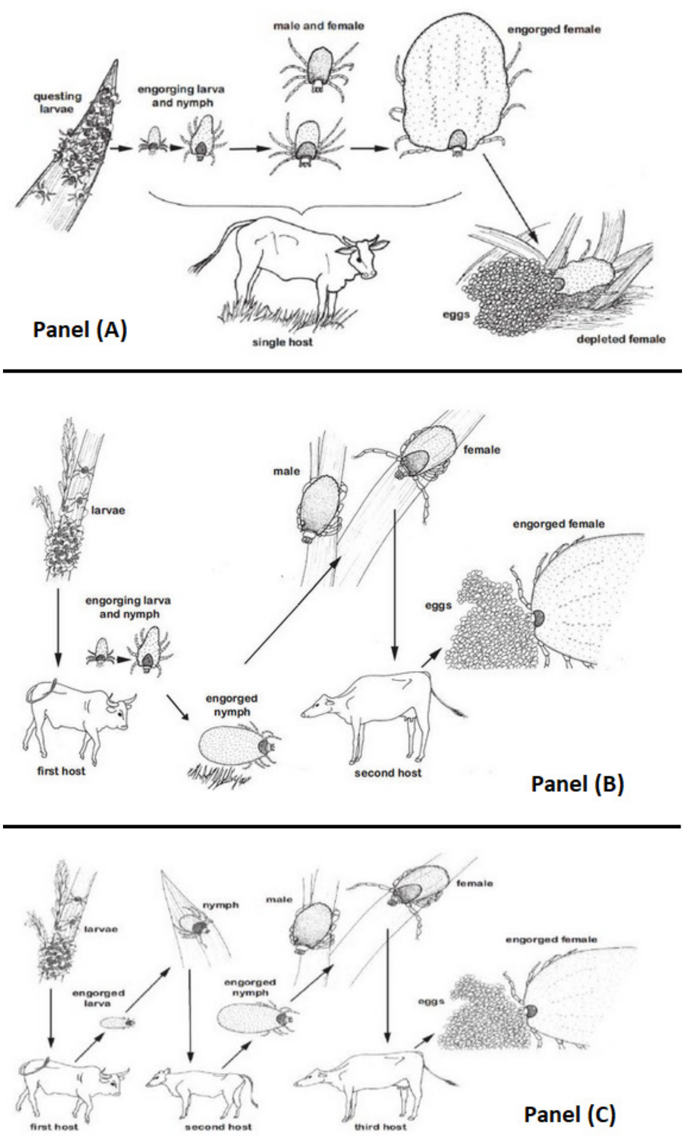

Figure 1: *Different species of Rhipicephalus with dissimilar life cycles; one-host (Panel A), two-host (Panel B), and three-host (Panel C) life cycles (rearranged from Alemayehu, 2018).*

Admittedly, common records file huge economic losses in both meat and milk production of livestock due to tick pests and TBDs. Hence, a brief background for the different life cycles of tick pests can not only demonstrate their key contribution in these losses but also help in grasping their economic importance from the standpoint of pest management. Their species are so serious that they may have one-, two-, or three-host life cycle as illustrated in the panels (Figure 1). For the two- and three-host life cycle, the animal hosts are not always the same species/individual but completing the cycle is contingent on availability of the host(s). Surprisingly, ticks favor a different animal as a host at each life stage (Iowa State University, 2023). Because ticks are obligate blood-feeders, they suck blood of their animal hosts for food and development which leads to general weakness and low productivity of the host animal. Ticks-life cycle comprises egg, the 6-legged larva, and 8-legged nymph and adult; male/female (Alemayehu, 2018). Except the eggs, all these life stages can climb onto an animal host to feed and develop. In some cases, the male remains on the host and attempts to mate with many females while feeding.

Improving methodology for priming EPNs

Significant components of the current advanced knowledge should be exploited for successful tick control. As adequate sampling and extraction techniques can frequently widen the available EPN gene pools (Baiocchi *et al*., 2017), improved sampling and extraction methodology should be followed. A rational sampling and extraction technique could detect high EPNs-positive samples where EPNs were recovered from all the sampled groves. This technique was based on integrating four factors. These factors engaged favorable sampling method, selecting the sampled site, suitable sampling season/time, and multiple- not single- baiting cycles for nematode extraction (Abd-Elgawad, 2020). Surely, related factors as handy funding, experience, and objective often dictate the chosen methodology for EPN detection and capacity of sampling, extraction, and identification techniques. For instance, insect-baiting methods could be applied in developing countries where alternative molecular techniques are still frequently unaffordable. Abd-Elgawad (2021b, 2024) addressed merits/demerits of the commonly used ones; that is, binocular vision, baiting methods, and polymerase chain reaction (PCR)-based techniques. These latter could provide fine-scale taxonomic resolution to distinguish closely related EPN species and consequently differentiate EPN phenotypes (Dritsoulas *et al*., 2021). Such phenotypes are necessary for the best EPN-host matching. Remarkable examples of them are EPN reproductive potential, insect host specificity, habitat adaptation, and persistence.

Obviously, these methods must be screened and improved to select the most appropriate one(s) for the existing variable(s). In this respect, Dritsoulas and

Duncan (2020) compared four common procedures to recover mesofauna including natural enemies and competitors of EPNs from soil. They found sucrose centrifugation method as the most effective one for recovering microarthropods. While their study could boost the taxonomic coverage of the existing fauna, it suggested sucrose centrifugation approach also to study EPN interactions with such subterranean competitors and natural enemies. Under their favorable conditions, growing population levels of EPN antagonists as a reaction to inundative release of EPNs might not achieve the required biocontrol of the pest. On the contrary, these conditions might temporarily reduce both EPN population densities and pest mortality severely (Duncan *et al*., 2003). Generally, fluctuations of EPN population levels are impacted by density-dependent growth by EPNs-antagonist populations, EPN species combinations, and/or availability of insect hosts (Wu and Duncan, 2022). Therefore, certain extraction technique may unravel the complexity linked to different biotic/abiotic variables in specific EPN setting. Hence, related research should investigate the impacts of these variables on a case-by-case basis (Abd-Elgawad, 2022b). Such a methodology will authorize us to track and enhance EPN biocontrol potential based on the existing components. It enables stakeholders to optimize the factors needed for the best EPN-host matching within integrated pest management (IPM) plans. Furthermore, common counting of typically high population levels of EPNs and related microorganisms are often tedious and time-consuming. Hence, automated counting of such population densities using high-throughput image processing is comfortable (Holladay *et al*., 2016). It can also expedite workflows for EPN processing with superior precision in counting. Abd-Elgawad (2024) recently reviewed additional aspects of sophisticated techniques that are currently presenting the state-of-the-art knowledge for general processes of EPNs.

Extending the spectra of EPN activity to pests of domestic animals

A limited spectrum of EPN activity could contribute to ecological and non-target safety, but even this range of EPN performance against pests has yet to be fully exploited. Initially, an ideal EPN-host matching via selecting the perfect nematode species/strain is also imperative to achieve the optimal biocontrol tactics/strategies against pests of domestic animals. The related biotic and abiotic factors must also be favorable. For instance, soil texture and moisture (Campos-Herrera *et al*., 2013), salinity (Nielsen *et al*., 2011), mulching/adding organic matter (Samish *et al*., 2008; Hussaini, 2017), and pH (Campos-Herrera *et al*., 2019) could alter EPN population densities in soil of pastures and grasslands used for raising livestock. Such a shift in EPN levels usually occurs either directly or indirectly by influencing EPN hosts and/or enemies (Campos-Herrera *et al*., 2019). These basics should operate as backgrounds for the transformational technologies required for adopting further share of EPN performance in controlling tick pests. Subsequently, relevant strategies should also focus on expanding commercial EPN products with such reliable biocontrol attributes into novel horizons; i.e., to penetrate/spread into tick control-related markets. Ultimately, biocontrol of related pests and diseases infesting domestic animals should rank high under favorable settings of EPNs. Even so, because these technologies are still being explored, tested, or used on a small scale, suffice it to mention a few examples vividly expressing their counterparts.

Examples and methods for effective biocontrol of ticks by EPNs

More pests and diseases of farm animals have recently begun to receive escalated attention for their biocontrol via applications of EPNs or the bioactive compounds of their mutualistic bacteria in developed countries (Filgueiras *et al*., 2022; Iowa State University, 2023; FAO, 2024). Also, numerous developing countries (e.g., in the Middle East and Africa) are trying to catch up with safe and effective pest control systems for environmentally friendly economic growth (Elhady *et al*., 2024). Within this context, the transformational step-change of expanding EPN products to target livestock pests/diseases is required worldwide. This is especially important as recent research results demonstrate the possibility of their achieving incremental gains in relevant market share. Numerous arthropod pests that attack and inflict severe diseases on many domestic animal species worldwide are being documented as quite EPN-susceptible hosts (Abdel-Ghany *et al*., 2024; Albogami, 2024). Also, from the aforementioned rationale, use of EPNs bodes well as favorable and benign alternative for treatment of ticks-infested animals. This does not negate the fact that there are differences in the degree of susceptibility regarding tick stages and species to the tested EPN species/strains. Lately, *H. bacteriophora* proved to significantly reduce *R. microplus* larval populations in infested pastures (Filgueiras *et al*., 2022). These populations comprised *R. microplus* engorged females from various geographical locations with different tick-body weights. Likewise, the brown dog tick, *R. sanguineus* Latreille (Acari: Ixodidae), is a key ectoparasite of dogs mainly but can also infest livestock, wild ruminants, and even humans. It is well-documented as the most widespread tick globally and a well-recognized vector of numerous pathogens affecting dogs and sometimes humans. As other tick species, *R. sanguineus* can not only cause anemia but also predispose the infested tissues of the animal to secondary bacterial, viral, and/or protozoal infections. Common chemical treatments against *R. sanguineus* are unsafe for cats/children, while household furniture should be deemed (Dantas-Torres, 2010). Like other similar EPN tests on various hosts, in vitro assessment of the EPN effect on *R. sanguineus* showed that its biocontrol depends mostly on the used EPN species/strain, the IJ concentration, and the insect-life stage with few exceptions (Kaaya *et al*., 2000). Various levels of tick mortality were observed for the different insect-life stages. Interestingly, the EPN species tested could achieve 100% mortality of tick-engorged females at 9 days post-exposure (Abdel-Ghany *et al*., 2024). Hence, it is timely to apply EPNs when abundant ticks of the engorged female stage are found to get the best effective results under actual conditions. These engorged females always seek moist environment in soil to avoid solar radiation like those habitats that are ideal for EPN biocontrol activity. Briefly, the ecological terms fully suit both the *R. sanguineus* engorged females at the oviposition time and the insecticidal nematodes.

Such a full percentage of pest mortality requires optimizing EPN delivery and persistence in soil to achieve the best effect on other tick species, to repeat this efficiency in other cases. It calls for providing optimal conditions for the biocontrol under field conditions too. At the pre-infesting stage, tick species usually live on/near soil surface and often crawl onto its vegetation to wait for their animal hosts to pass by. Meanwhile, many EPN species can reside on the same places as their natural habitats. Thus, larvae, nymphs, and adults of susceptible species in tick genera such as *Haemaphysalis*, *Rhipicephalus*, and *Ixodes* can easily be challenged by the nematodes. Other chances of EPNs to practice their biocontrol activity on a different variety of animal parasites are represented by adult ticks of species in the genera *Hyalomma* and *Amblyomma* (Albogami, 2024). As active hunters, species of these tick genera crawl/run across the ground after nearby animal hosts; giving additional room for both ambusher and cruiser nematodes to find and invade them. Furthermore, to optimize EPNs according to the settings (corrals, stables, cowsheds, pastures, grasslands, ranches, grazing areas, and ranches) of the target insect stage(s), EPN applications should be fitly employed. For newly hatched subterranean tick larvae and older larvae that climb to tips of vegetation to attach to passing livestock hosts, IJ suspension can be sprayed as soil treatment or delivered via irrigation systems (Shehata *et al*., 2021). That is because EPNs inhabit the soil as natural residence where 95% of the tick population is found (Samish *et al*., 2008). Collectively, ticks-infested habitats like pastures and grasslands are favored by EPNs in such settings.

If not controlled in/on soil, the tick grabs onto the host and crawl over its skin to find a proper place to attach and feed (Alemayehu, 2018). In this case, spraying adequately formulated EPN suspension on the proper/infested sites of animal skin would still be good alternative tactics. However, direct spray of the nematodes on the ticks-infested animal requires protection against common stresses such as low moisture or/and solar radiation. In this case, the efficacy of IJs is linked to the time of permanency of the nematode formulation on the animal's body as they need enough time to find a tick host. This case needs several precautions. First, it is imperative to identify the best EPN formulation that suites such settings. Aquino-Bolaños *et al*. (2019) could test and identify beneficial formulations of vegetable oil emulsions that prolonged the EPN survival at ambient temperature and boosted their infectivity on ticks in both laboratory bioassays and under field conditions. They tested emulsions of five vegetable oils that are known to be compatible with three EPN species (*H. bacteriophora*, *S. websteri*, and *S. carpocapsae*) and possess superior levels of viscosity and evaporation compared with water. Consequently, these properties could delay IJ desiccation and enhance their biocontrol activity against the tick pest (*Ixodes scapularis* Say) on the animal's body. Having optimized the time of contact and the oil type, they recommended the use of *Steinernema websteri* emulsified in *Juniperus virginiana* oil as the best alternative option to unhealthy chemicals used for controlling ticks. The latter suspension achieved the highest mortality, 89%, of *I. scapularis* at 96 h postapplication (Aquino-Bolaños *et al*., 2019). Second, a wise spray of EPNs should mostly be on areas of the animal's body where ticks are expected to be easily found. These may include the ears, neck, and the perianal zone where the skin is thinner; making the tick feeding more comfortable. Third, continuous developments are still needed to turn theory into practice or science into technology. This is recently manifested in a smart sprayer for direct application to animals and the surrounding feeding area could be developed for tick control. It only operates/sprays when cameras detect the animal at the feeding station so there is no waste of EPNs (Goolsby *et al*., 2018; Goolsby and Shapiro-Ilan, 2020). Of special interest is its application in the remotely managed sprayer dedicated for controlling *R. microplus* infesting nilgai antelope (Shapiro-Ilan and Goolsby, 2021). Also, other applications of EPN formulations to effectively control insect pests on animal skin are being earnestly refined. Barricade® (a sprayable fire gel) mixed with the *Steinernema riobrave*-water solution at 1 and 2% rates and applied to cowhides could protect the nematodes from ultraviolet and desiccation too (Shapiro-Ilan and Goolsby, 2021). Consequently, *S. riobrave* biocontrol efficacy was significantly enhanced on cowhides set out of doors for 30 and 60 min of sunlight relative to the control (without Barricade®). Promising results for controlling ticks infesting animals by EPNs are shown in Table 1.

A full beneficial spectrum of bioactive compounds from EPN-mutualistic bacteria against livestock pests should also be exploited and expanded. These compounds and their potential as BCAs were reviewed for various bacterial species in the genera *Photorhabdus* (Abd-Elgawad, 2021a) and *Xenorhabdus* (Abd-Elgawad, 2022a). For veterinary management, EPNs or the bioactive compounds of their symbiotic bacteria could safely and effectively control several serious pests of livestock such as *Hyalomma dromedarii* (El-Sadawy *et al*., 2008a, b), *Eimeria* oocyst (El-Sadawy *et al*., 2009), *Argas persicus* and *Rhipicephalus annulatus* (El-Roby *et al*., 2018), and *R. microplus* (Singh *et al*., 2018). Also, bacterial species of *Photorhabdus* and *Xenorhabdus* have recently proved to release larvicidal compounds against other key pests such as *Culex pipiens* L. (Yüksel *et al*., 2023) and *Aedes albopictus* Skuse (Touray *et al*., 2024). The latter insect can transmit filarial nematodes in the genera *Serratia* and *Dirofilaria* that severely affect domestic animals. Applications of these bacteria and/or their bioactive compounds against such a variety of veterinary pests will rely on our sound grasping of their capacities/attributes and interactions with the relevant biotic and abiotic factors. Similar to EPNs, a variety of techniques should be sought regarding adequate application of these bacteria, their enhanced shelf life, economical mass production, and knowledgeable integration with supplemental agricultural inputs.

Other transformational step-changes to boost their uptake

Some products to control insect pests and insect-borne diseases are being developed using EPNs (Koppenhöfer *et al*., 2020) and their symbiotic bacteria (Park *et al*., 2016). However, boosting EPN uses against pests of animals and crops will require lightening growers and stakeholders with their sound uses, biocontrol spectra, non-side effects, and

Table 1: *Examples of ticks-infested animals and their control by entomopathogenic nematodes.*

Targeted tick species	Infested animals	Nematode species	References
Rhipicephalus microplus	Nilgai antelope	*Steinernema riobrave*	Goolsby and Shapiro-Ilanb, 2020
Rhipicephalus sanguineus	Mainly dogs, occasionally livestock, ruminants, humans	*Heterorhabditis bacteriophora* HP88 and *Steinernema* sp. SII	Abdel-Ghany *et al*., 2024
Ixodes scapularis	Dogs and others (e.g., white-tailed deer)	*S. websteri*	Aquino-Bolaños *et al*., 2019
Rhipicephalus (Boophilus) microplus	Calves	*H. bacteriophora*	Filgueiras *et al*., 2022
Argas persicus and Rhipicephalus (Boophilus) annulatus	*A. persicus* on domestic fowl (e.g., chickens, ducks, geese). *R. annulatus* on cattle	*Heterorhabditis* spp. (isolates A4 and 10K)	El-Roby *et al*., 2018
Amblyomma americanum	Sheep	*S. riobravus* and *S. feltiae*	Kocan *et al*., 1998
Hyalomma dromedarii	Mainly Camels; also other animals	*Heterorhabditis* spp.	El-Sadawy *et al*., 2008a, b
Rhipicephalus microplus	Cattle	*H. baujardi* (isolate LPP7)	Mendonça *et al*., 2019

merits to secure safe foods. They should absorb advanced methodologies for their full gains. Marketing support for utilizing the EPN or symbiont products is also needed to expand their commercialization (Coupland *et al*., 2017). Also, a paradigm shift to perfectly perceive EPN efficacy based on partial successes/failures and their costs is still needed. The paradigm should be quite different from a chemical pesticide model that is based on cheap and stable products with easy application to major crops. More materialized examples of notable success with astute marketing and usage of EPNs are required to achieve a shift in mindset away from the current chemical model (Nagesh *et al*., 2017). Enlightening growers and sales representatives with sound ways and handling of EPNs and their application should represent a priority for attracting end-users adoption. Incentives for pesticidal registration of low-risk materials like EPNs especially for their compulsory employment in IPM practices should be enacted regulations. They should replace frequent hobbling by bureaucratic and strict regulation.

Meanwhile, the above-mentioned focus on improving EPN efficiency and reducing cost should be supplemented. Reducing application rate via genetically upgrading IJ persistence and virulence could target two birds (EPN cost and efficiency) with one stone (Ehlers *et al*., 2022). The authors used only 1 billion *H. bacteriophora* IJs/ha at sowing instead of the old common standard 2.5 X 10^9 IJs/ha (Gaugler, 2002) to effectively control *Diabrotica virgifera virgifera* on maize. Also, merits and demerits of the three main mass production methods of EPNs that have notable role in their marketing success globally are quite apparent (Shapiro-Ilan *et al*., 2023). Therefore, work must continue to avoid the disadvantages of each method and maximize its advantages. For instance, optimizing *in vivo* production may increase cost efficiency as *Galleria mellonella* L. is most commonly used for *in vivo* culturing of EPNs. Recently, a cheaper diet than common one is composed via cost-effective ingredients for abundant mass production of *G. mellonella* (Suyal and Pandey, 2024). On the other, in vitro mass production focusing on highly qualified EPN biocontrol activity can reform EPN impact and also reduce costs (Ehlers *et al*., 2022). Likewise, cost-effective mass culture could be obtained for their mutualistic bacteria. The produced *Photorhabdus* spp. showed enhanced insecticidal activity as their related traits could be maintained during growth/metabolic phases of their inexpensive culturing (Keskes *et al*., 2021). Such tremendous strides made in mass production of EPNs and the symbionts should be widely expanded. This will need further elevation in academic-industry partnerships.

Because of the shortest time that augmentation (inundative) biocontrol method goes through, relevant biotic/abiotic factors should be harnessed. These include the best EPN-host matching, production practice, formulation, ambient factors like temperature/moisture, and timing for EPN survival and efficacy (Koppenhöfer *et al*., 2020; Shehata *et al*., 2021; Tarasco *et al*., 2023). Although augmentation is the most used EPN biocontrol method, we should earnestly advance the use of the two other methods; i.e., classical and conservation biocontrol ones. Much directed surveys are needed for settings/environments in which the EPNs will have to develop the required merit or trait for classical biocontrol. For conservation biocontrol, perennial systems seem more favorable for this method than annual crops due to relative soil stability. Even so, perennials such as Florida citrus groves relied on specific scenario to control the root weevil *Diaprepes abbreviatus* L. (Duncan *et al*., 2013). In these groves, EPN diversity and efficacy was driven by variables linked to water content and soil texture. These variables could modulate the soil food webs for efficient conservation biocontrol. Campos-Herrera (2022) speculated that with a notable effect linked to the crop, introducing ecological structures might help to back the EPN persistence and consequent conservation biocontrol. She reported that natural cover crops grown within lines in vineyards favored the EPN occurrence/persistence relative to classical tillage practice. Hence, the soil-multitrophic interactions affecting EPN efficacy should be better grasped to offer the best habitats that boost their conservation biocontrol under different scenarios. When multiple insect species are found in grazing areas such as ranches and pastures, evaluating EPN biocontrol based on targeting a single insect species could be misjudging. Application value of EPNs/symbionts should include controlling all the existing host species. In this case, considering the whole scenario will save costs (Kepenekci *et al*., 2018) and may support the conservation biocontrol approach. Therefore, this approach in grazing areas has yet to be further explored.

Conclusions and Recommendations

Various species of tick pests that parasitize domestic animals proved to be reliably controlled by the safe insecticidal nematodes. In contrast, malpractices in applications of excessive acaricides against ticks infesting domestic animals have resulted in their common resistance to such unhealthy chemicals. Practically, EPNs can be a useful tool for controlling tick pests, as they reside in sites that are natural habitats to highly susceptible tick stages such as the engorged females. Thus, EPNs could be used to effectively suppress the tick populations, though the nematodes do not utilize their natural reproduction cycle within tick cadavers. Notably, differences in susceptibility of tick species/stages to the tested EPN species/strains are crucial. Matching EPN species/strain in terms of infectivity and virulence to best fit specific tick species/population could improve the tick biocontrol tactics. Nevertheless, EPN usage may be restricted to defined ecological terms due to their sensitivity to low humidity as well as elevated silt/clay fraction in soil, and/or mulching/manure concentration. Therefore, in order to optimize strategies to control tick pests, EPN procedure, formulation, and application tactics should be continuously refined to meet the intended settings and tick species/stage. These may include widening available EPNs-genetic pools, detecting novel strains yet to be found, assembling genetically manipulated strains, and/or developing EPN formulations and application techniques that can be successfully used against ticks. The smart sprayer has already been developed as successful tool for tick control. It only sprays the nematodes when cameras detect the animal at the feeding site so there is no waste of EPNs. Barricade® (a sprayable fire gel) combined with the *S. riobrave*-water suspension could be applied to cowhides to protect the nematodes from ultraviolet and desiccation. Also, using suitable oil emulsion of EPNs could prolong their efficacy against ticks. Further innovatory studies of the interaction between EPNs and tick pests under actual settings are required and could prove to be so useful.

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Novelty Statement

The study aims to provide evidences for the possible commercial expansion of EPN products to control tick pests of domestic animals. Their integration into other pest management schemes offers novel strategies to avoid the problems resulting from developing resistance of the ticks to acaricides.

Abbreviations
BCAs, Biocontrol agents; EPNs, Entomopathogenic nematodes; FAO, Food and Agriculture Organization of the United Nations; HTS, High-throughput sequencing, IJs, Infective juveniles; IPM, Integrated pest management; PCR, Polymerase chain reaction; PPNs, Plant-parasitic nematodes; qPCR, Real-time quantitative PCR; TBD, Tick-borne diseases; VOCs, Volatile organic compounds.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests
The author has declared no conflict of interest.

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