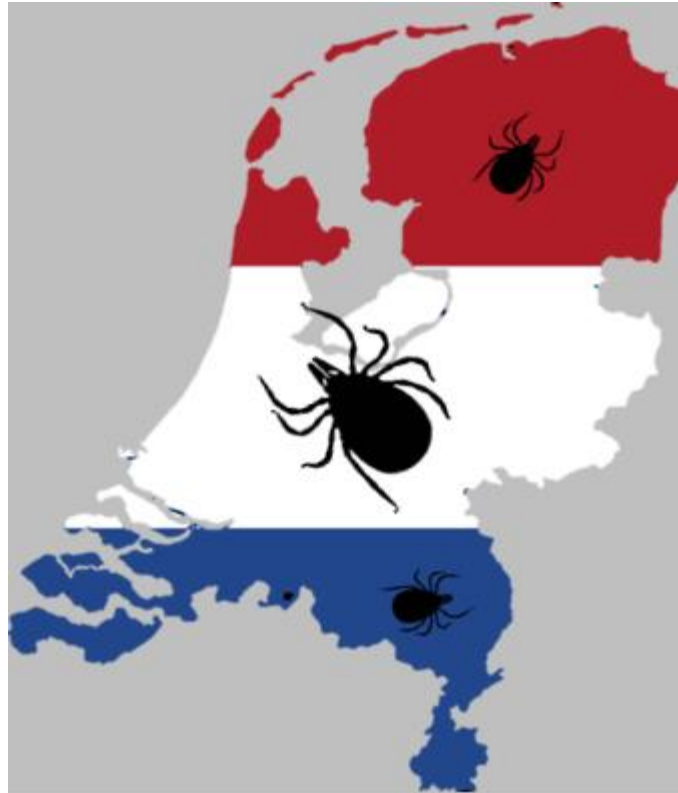


Ticks in the Netherlands: from the perspective of owners of companion animals



Master thesis Veterinary Medicine

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PREFATORY NOTE

During the master's program of Veterinary Medicine at the University of Utrecht, all students must conduct a research internship and a master thesis. This report is the master thesis of the research internship carried out by S. van Emden at the department of Infectious Diseases and Immunology, under supervision of the division Clinical Infectiology of the University of Utrecht and the Institute of Risk Assessment.

Research was conducted to gain more insight in Dutch owner's knowledge about ticks present on companion animals and tick-borne diseases, the used tick control methods and the population proportions of tick species in the Netherlands. In addition, research was conducted to gain insight in the knowledge of dog and cat owners about tick-borne diseases. Data for this research was obtained among pet owners and veterinarians at the same time. Data about prevalence of tick borne diseases, recommended prevention methods and a possible new tool for removing ticks on dogs can be found in the report of D. Spreen, another student of the faculty of Veterinary Medicine.

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ABSTRACT

Objectives: To gain further insight in the current situation regarding ticks in the Netherlands among Dutch dog and cat owners

Method: Ticks found on dogs and cats in the Netherlands in 2019 during the months June up until October, were send in through the Bayer tickscanner application. From these ticks, the tick-species was determined. Additionally an online questionnaire among Dutch dog and cat owners was conducted.

Results: Through the application 811 ticks were reported in the period from June '19 up and including October '19. Of these, 554 ticks from dogs and 146 ticks from cats were actually submitted for analysis. From the submitted ticks, 655 were identified as *Ixodes ricinus* ticks (93.6%), 33 as *Ixodes hexagonus* (4.7%), 11 as *Dermacentor reticulatus* (1.6%) and 1 as *Rhipicephalus sanguineus*.

In the questionnaire, general tick-related questions were posed to 178 Dutch pet owners. The survey showed an association in which owners with knowledge about tick-borne diseases are more motivated to use tick preventatives for their dog or cat. Bravecto™, with fluralaner as active component, is the most frequently used treatment (22,7%) among the participating Dutch pet owners. Owners with knowledge about tick-borne diseases, also seem to ensure a longer efficient level of tick-prevention for their pets and are more likely to use a proper device for tick removal. Furthermore, owners that buy tick prevention treatment at a veterinarian practice, have significantly more knowledge about tick-borne diseases. Owners that buy tick prevention treatment elsewhere (internet or pet shop), buy unlicensed products significant more often.

Conclusions: Dutch pet owners with knowledge about tick-borne diseases are more motivated to use tick prevention for their dog or cat. Bravecto™ is the most frequently used treatment (22,7%). Owners with knowledge about tick-borne diseases, ensure a longer efficient level of tick-prevention for their pets and use a proper device for tick removal more often. Owners that buy tick prevention treatment at a veterinarian practice, have significantly more knowledge about tick-borne diseases. Owners that buy tick prevention treatment elsewhere (internet or pet shop), buy unlicensed products more often. There is still room for improvement with regard to educational advice provided by Dutch veterinarians about the importance of tick prevention in companion animals and the possible consequences if this is not done properly.

INTRODUCTION

TICKS IN GENERAL

Ticks are ectoparasites belonging to the class of the Arachnida. Besides mosquitoes, ticks are the most important vectors for a great number of pathogens which affect human and animal health¹. Ticks can be divided into 3 families; Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae². This last mentioned tick family consists of only 1 species, *Nuttalliella namaqua*, which is mainly seen on poultry in Africa³. Soft ticks are present in the Netherlands, but feed primarily on birds. Since this thesis focuses on dogs and cats, the soft ticks are disregarded. The family Ixodidae is subdivided into 7 genera; *Amblyomma*, *Anocentor*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*². In Europe, the *Ixodes* ticks are particularly of interest, due to their extensive spread in ecosystems and their broad range of transmissible pathogens which affect both humans and animals². On global basis, ticks transmit a larger number of pathogenic organisms than any other arthropod vector group. Because of this, ticks are among the most important vectors affecting humans, companion animals and livestock⁴. In companion animals, concerns regarding animal welfare and pathogen transmission are the most important. Additionally, with companion animals living close to humans, an efficient tick prevention method is beneficial to minimize the theoretical risk for owners for contracting tick-borne diseases as well⁵.

MORPHOLOGY HARD TICKS

Ticks are the largest group among the Acari, which is a taxon of Arachnids. Unfed adult ticks are 2-7mm of size, while fed females are 8-30mm of size. All Acari are unsegmented, oval and dorsoventrally flattened when not fed. The nymph stage and the adult stage have 4 pairs of legs, while the larvae have 3 pairs of legs. All ticks have one double claw (pulvillus), located on the most distal limb of the legs. The well-developed pullvilli, enable the tick to walk on smooth skin and vertical surfaces. Ticks have a sensory organ, the Haller's organ, in the tarsi of the first pair of legs. The Haller's organ is especially of importance during infestation of the host, by exposing this organ, ticks can perceive chemical (volatile substances) and physical (temperature, vibrations) signals from hosts. All stages of hard ticks consist of a dorsal, cuticular shield, the scutum. In female hard ticks the scutum covers up to one third of the body, this in contrast with male hard ticks where the scutum covers the entire body. The scutum prevents the male hard ticks to imbibe a large blood meal. Male ticks are able to take up a maximum blood meal of 2 times the bodyweight, whereas a female tick is able to take up 100 times her bodyweight^{2,6}.

The mouthparts of the Ixodidae consist of palps and chelicera fused to the basal capitulum (Figure 1.1). The hypostome is a mouthpart which is used to pierce the skin of the host. The chelicerae are also part of the piercing apparatus. For spatial orientation, ticks use their palps. In both sexes, the genital opening is located between the hind legs on the ventral side. The anus is located ventrally near the posterior. The anus is enclosed by the anal groove, which is an important taxonomic feature in ticks⁶.

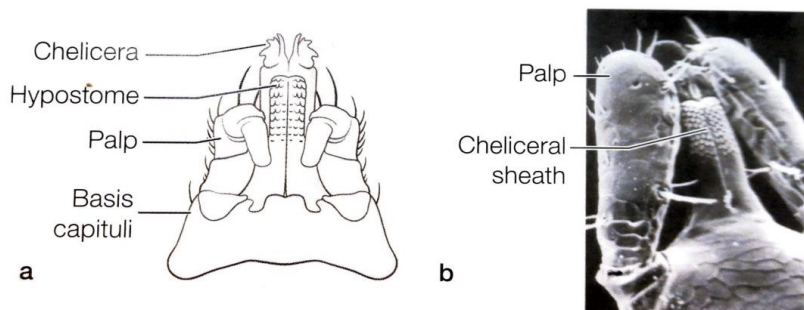


Figure 1.1 Mouthparts of Ixodidae⁶

LIFE CYCLE AND EPIDEMIOLOGY OF HARD TICKS

Hard ticks develop through four stages: egg, larva, nymph and adult. Between each stage, blood must be ingested to induce molting. Depending on the number of hosts involved during the development of the tick, a distinction is made between 3-, 2-, or 1- host ticks. Most hard ticks are three-host ticks, they feed on three different hosts and drop off on the ground between each stage of the life cycle to molt. With two-host ticks, the larvae and nymphs remain on the same host. One-host ticks can complete the whole life cycle on only one host^{2,6}.

IXODES RICINUS (THE CASTOR BEAN TICK/SCHAPENTECK)

The life cycle of *Ixodes ricinus* is shown in figure 1.2. *I. ricinus* is a three-host tick. The blood feeding process in the larval stage lasts for 2-3 days, preferably on small mammals (e.g. mice) and birds. In nymphs, who prefer larger mammals (e.g. deer, foxes, dogs, cats, cattle and sheep), birds and reptiles, this process lasts up to 5 days and in adult females 1-3 weeks. Mating takes place on the host, after mating the female falls off the host and lays up to 3000 eggs. The total life cycle of *I. ricinus* lasts 2-3 years on average⁶. *I. ricinus* occurs throughout the most of Europe and parts of Asia and Africa⁶⁻⁸. In the Netherlands, Nijhof (2007) collected over 4000 ticks between July 2005 and October 2006. The majority of these ticks was identified as adults (67.6%) and nymphs (12.3%) of *I. ricinus* and larvae (9.0%) of *Ixodes* spp. Between April and September 2018, Jongejan (2019) collected 1273 ticks from dogs and cats in the Netherlands. Within this research, *I. ricinus*, was the most prevalent species (90%) detected on dogs and cats.

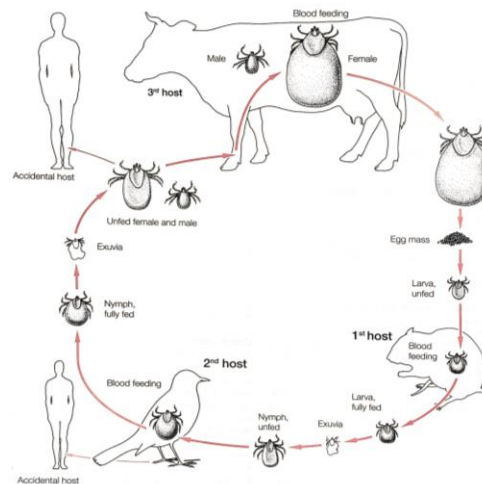


Figure 1.2: Life cycle of *Ixodes ricinus* ⁶.

IXODES HEXAGONUS (THE HEDGEHOG TICK/EGELTECK)

Ticks from the species *Ixodes hexagonus* are also 3-host ticks and have an average life cycle of 1-2 years⁹. In contrast to *I. ricinus* mating does not take place on the host. Males remain in host's resting places and when an engorged female falls off the host, mating can occur⁶. The occurrence of *I. hexagonus* spreads over Europe and Northwest Africa^{1,8}. In the research of Nijhof (2007), the prevalence of *I. hexagonus* was 7.6%. In the research of Jongejan (2019), *I. hexagonus* was the second most seen tick species on dogs and cats with a prevalence of 7.3%.

DERMACENTOR RETICULATUS (ORNATE COW TICK/ VLEKKENTECK)

Dermacentor reticulatus ticks are also three-host ticks, with an average natural life cycle of 1-2 years⁶. The *D. reticulatus* tick is a palearctic species with a highly focal distribution pattern^{9,10}. It is present in the temperate zones of Europe and Asia. This distribution is most probably associated with the climatic profile of Europe. The minimum temperature in which questing behavior is seen, varies between different studies. Karbowski (2014) describes the absence of *D. reticulatus* in areas with mean winter temperatures of 0 to 5°C. However, in research performed by Mierzejewska (2017) *D. reticulatus* ticks were found in areas with temperatures below 5°C, questing behavior was even observed in areas with temperatures varying from 0.7°C to -0.1°C. The expansion of this tick species may be the effect of climate change, which has caused the mean temperatures worldwide to increase. Climate change has caused the vegetation season to lengthen and the number of wet days to increase. As a consequence, northern Europe is becoming wetter and warmer. Furthermore, human influence is also causing the spread of *D. reticulatus* by changes in the use of

agricultural land, environmental protection and globalisation¹¹. In an article published by Nijhof (2007), *D. reticulatus*, which was not known to be endemic in the Netherlands, was collected from six different locations from the vegetation in this country. This confirmed the presence of *D. reticularis* populations in the Netherlands with a prevalence of 1,7%. In an article presented by Jongejan (2015), 1368 *D. reticulatus* ticks were collected from different locations in the Netherlands. The researchers conclude that *D. reticulatus* is spreading to novel areas in the Netherlands. In an article by Jongejan (2019) the prevalence of *D. reticulatus* is 2,4%. Unfortunately, confidence levels are missing in these researches, which makes it hard to make a comparison between both researches and the results from this research essay. The aim of this research is to make an estimation about the current situation in the Netherlands. For this, the population proportions of different ticks will be determined. Thereby this research conducted the following hypothesis: The current population proportion of tick species found on dogs and cats in the Netherlands between June and October 2019 corresponds with the prevalence mentioned in the article published by Nijhof (2007).

INFESTATION OF THE HOST

During infestation of the host, roughly four different stages can be seen: activation of the tick, appetite behavior of the tick, contact of the tick with the host which is followed by attachment of the tick to the host. Both, hard ticks (e.g. *Ixodes* spp.) and soft ticks (e.g. *Argasides* spp.) are exposed to a host's defense system like acute inflammation and hemostasis after penetration of the host's skin. Hard ticks, however, must also act against chronic inflammation responses, which include cellular and humoral immunity¹².

Environmental beneficially temperatures and humidity conditions, stimulate the tick to leave their ground hiding place and climb up the vegetation. Hard ticks are 'ambushers', which means that they use vegetation along tracks and paths to get on a host. By exposing the Haller's organ, ticks can perceive chemical (volatile substances) and physical (temperature, vibrations) signals from hosts. The chemical signals are detected by receptors: CO₂ is detected by 2 antagonistic receptors whereas H₂S, ethyl mercaptan, dimethyl sulfoxide and a variety of sexual pheromones are detected by other highly sensitive receptors. CO₂ detection is of importance when searching for a host, whereas the other mentioned substances are of importance in tick to tick communication. When the host is identified, the tick approaches and contacts the host. When contact is made, the chelicerae and palps are used to identify optimal sites for blood feeding. The chelicerae are used to cut through the epidermis and a part of the epidermis. *Ixodes* ticks have long chelicerae with a long hypostome, which are deeply attached into the dermis of the host⁶. In *Dermacentor* ticks attachment is obtained through the hypostome and by forming a cement cone. Also, the saliva creates a counteractment for the hemostatic, inflammatory and immune reactions of the host¹³. The preparatory phases mentioned above take up to one day, without feed intake. After this, the blood-sucking process, which consists of 2 phases, follows. In the first phase (2-5 days in pre-adult stages, 5-10 days in females) ticks obtain cells and fluids from their host. By doing this weight is slowly gained. The second phase lasts 12-24 hours. In this phase blood is ingested, which is concentrated by excretion through the salivary glands. This phase is completed by detachment from the host. Skin lesions in hosts correspond to the size of the mouthparts, which are small in evolved ticks (*Dermacentor* spp.) but can be large in primitive ticks (*Ixodes* spp.). Lesions caused by nymphs or larvae are usually small and often stay unnoticed. At the location of the lesion there may be various changes: hyperaemia, erythema, ulceration, bleeding or necrosis. A chronic inflammation involving thickening of the skin, eosinophils, formation of nodules or granulomas may follow⁶.

TRANSMISSION OF PATHOGENS

In the following text, the pathogens mentioned are used as examples. Not all of the mentioned statements are applicable for all pathogens.

Already during the appetence and contact phase, infective stages of some pathogen species develop in the salivary glands⁶. In research performed by Alekseev evidence is provided that tick-borne encephalitis virus and *Borrelia burgdorferi* accumulate in the cement plug within the first few hours after attachment to the host. Even if the tick is extirpated without the cement plug soon after the attachment, transmission was not prevented. For TBE virus and Powassan virus transmission took place within one hour of attachment and for *B. burgdorferi* after 20-22 hours^{14,15,59}. After infestation of the host, dynamic, multi-directional interactions occur between tick, host and transmitted pathogens^{16,17}. The interaction is shown in figure 1.3. Environmental circumstances in the midgut of the tick can interact with ingested tick-borne pathogens, which invade the salivary glands after migrating through the hemolymph. Pathogens that proliferate in the salivary glands can bind salivary proteins on their surface, which facilitates infection of the host. Tick saliva consist of a rich species-specific spectrum of >30 components, interfering with the physiology of the host. Immunological defense mechanisms (e.g. alternative complement pathways, phagocytosis, NK cells, formation of oxygen radicals, effects of cytokines) of the host will be inhibited by several salivary components. The reduction of the inflammatory reaction in the host, protects the tick during the long blood-sucking phase and facilitates pathogen transmission. For example the tick saliva contains, apyrase, prostaglandins (PGE₂ and PGF₂), prostacyclin, and several anticoagulants, which counteract the hemostasis (platelet aggregation, vasoconstriction and blood clotting) mechanisms of the host. Other inflammatory reactions in the host (e.g. skin reaction and mast cell associated inflammation) are counteracted by cement, antihistamine activity and carboxypeptidase^{6,18-21}. All the described processes facilitate tick feeding and pathogen colonization of the tick, host or both.

Whether a pathogen is transmitted depends on multiple factors including duration of feeding by the tick. Research performed by Kahl (1998) showed that transmission of *Borrelia burgdorferi* sensu lato by *I. ricinus* needs at least 16 hours of blood feeding. Between different species of *Borrelia*, time to transmission may vary²². Research performed by des Vignes (2001), showed that for transmission of *Anaplasma phagocytophilum* by *I. scapularis* at least 24 hours of blood feeding is necessary. In other studies variable results were found, reported transmission times for *A. phagocytophilum* pathogens from *I. scapularis* to mice, ranged from less than 24 hours to greater than 40 hours^{23,24}. The transmission of *Babesia canis* by infected *D. reticulatus* needs between 8h and 24h of blood feeding¹⁵.

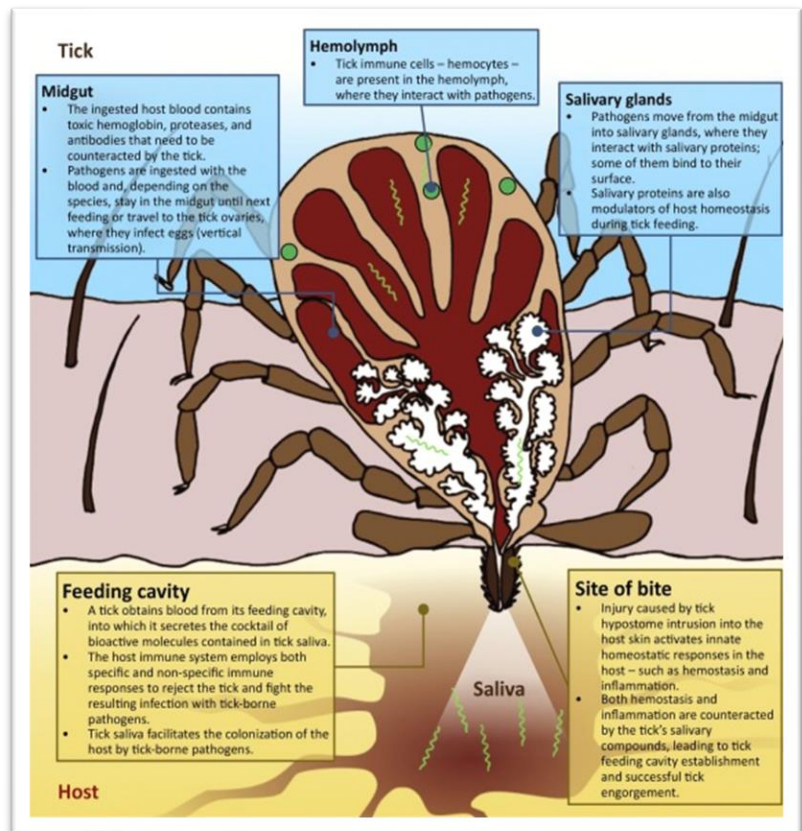


Figure 1.3: Tick host interaction¹².

In response to the physiologic changes that accompany prolonged tick feeding, genes responsible for pathogen replication and/or migration through the hemocoel and into the salivary glands are differentially expressed, resulting in the transition of a pathogen from a dormant state to a fully infectious form. As a result, a variable period exists (generally 12–48 hours) between tick attachment and pathogen transmission¹².

Pathogens can be transmitted by several pathways: stadia, transovarial, co-feeding and sexually. There are 3 types of stadia transmission: intrastadia transmission, stadium-to- stadium transmission and transstadial transmission. There are 3 types of stadia transmission: intrastadia transmission, stadium-to- stadium transmission and transstadial transmission. During stadia transmission a tick becomes infectious by a host and transmits the pathogen when feeding on another host. With stadium-to-stadium transmission the tick becomes infectious when feeding on an infected host, but the pathogen is only able to be transmitted to a new host after the ticks molts into the next life stage. With transstadial transmission at least 2 moltings are required before the pathogen can be transmitted to a new host^{6,25}. During transovarial transmission, the pathogen is transmitted vertically from the female tick to the eggs and thereby the larvae²⁵. In general, larvae are not infectious because they have to obtain pathogens during their first blood feeding. Only pathogens (e.g. *Babesia canis*) which can be transmitted transovarial can cause infectious larvae. When infected and non-infected ticks simultaneously feed adjacent to each other on a host, the non-infected tick can obtain pathogens that were inoculated by the former infected tick. Certain types of pathogens can also be transmitted during sexual interaction of male and female ticks, e.g. *Rickettsia* and African swine fever virus⁶.

PATHOGENS IN *I. RICINUS*, *I. HEXAGONUS* & *D. RETICULATUS*

In the Netherlands *I. ricinus*, *I. hexagonus* and *D. reticulatus* ticks are commonly seen. These ticks may be the carrier of various pathogens with the potential of generating serious human and veterinary diseases. An overview of pathogens is shown in table 1.1

Infection of ticks with *Borrelia* spp. can occur through stadia transmission. *Ixodes ricinus* can also be carrier of *Babesia divergens* and *Babesia microti*²⁶. Infection of ticks with *Babesia divergens* can occur through transovarial and transstadial transmission, however not all *Babesia* spp. can be transmitted transovarial. For example, transovarial transmission for *Babesia microti* is not confirmed. Infection of ticks with *Anaplasma phagocytophilum* and *Neoehrlichia mikurensis* only occurs through stadia transmission. *Rickettsia helvetica* can be transmitted transstadial within a generation, sexually and through co-feeding between ticks⁶.

Table 1.1: Pathogens in *I. ricinus*, *I. hexagonus* and *D. reticulatus*.
H = pathogens of importance in humans, A = pathogens of importance in animals^{6,26-29}.

Tick species	Pathogen
<i>I. ricinus</i>	<i>Babesia divergens</i> (H), <i>Babesia microti</i> (A), tick-borne encephalitis virus (H,A), <i>Anaplasma phagocytophilum</i> (H,A) and <i>Rickettsia helvetica</i> (H)
<i>I. hexagonus</i>	<i>Borrelia burgdorferi</i> (H,A), <i>Anaplasma phagocytophilum</i> (H,A)
<i>D. reticulatus</i>	<i>Rickettsia slovaca</i> (H), <i>Borrelia burgdorferi</i> (H,A), <i>Francisella tularensis</i> (H,A), <i>Babesia caballi</i> (A), <i>Babesia canis</i> (A) and <i>Theileria equi</i> (A)

ZOÖNOTIC RISKS

In human and veterinary medicine, ticks are relevant because they transmit pathogens which are responsible for vectors or reservoirs in the transmission diseases caused by bacteria (e.g. Lyme disease, Q-fever, dermatophilosis), by fungi, by protozoa (babesiosis and theileriosis) and by viruses (e.g. Crimean-Congo hemorrhagic fever, Powassan encephalitis, West Nile encephalitis, African swine fever (ASF), tick-borne encephalitis (TBE), Rocky Mountain spotted fever)³⁰. Over 100 arthropod-borne infections can be associated with 116 tick species (32 Argasidae and 84 Ixodidae). Since tick-borne diseases are a general problem in medical and veterinary medical clinical settings, the importance of the one health approach, which unifies biologists, environmental specialists, physicians and veterinarians in their disease management of zoonosis and communicable diseases, is alerted³¹. Wildlife and ticks themselves are the main reservoir of tick-borne pathogens of veterinary and medical concern. Domestic animals (e.g. dogs) can carry ticks that are infected with these pathogens. This does represent a zoonotic risk for humans³². However, when an infected tick is brought into a household by a dog, the tick will hardly ever detach and switch host. This makes the direct risk of pathogen transmission to humans minimal. Nonetheless, the introduction of pathogen infected nymphs and larvae into a household could become a zoonotic risk when the infected nymphs and larvae change into the next development stage. This is especially a problem in ticks with fast development times. Ticks of some species (e.g. *R. sanguineus*) may even establish an in-house (or garden) population³¹. Mulder (2013) even describes an unexpected large number of Dutch citizens reporting tick bites from their gardens (31%)⁵⁵. Another risk of human infection, are ticks that are crushed during removal from a pet animal. When the tick is crushed, salivary gland material might be exposed to wounds on the owner's hands which might create an opportunity of infection for certain pathogen species³³.

In a study performed by Nijhof (2007), 4298 ticks from companion animals were submitted by veterinarians between July 2005 and October 2006. All ticks were identified and screened by polymerase chain reaction (PCR) and reverse line blot (RLB) hybridization for the presence of pathogens. In *I. ricinus* ticks, infection with *Rickettsia helvetica* (24.7%), spirochetes from the *Borrelia burgdorferi sensu lato* group (7.2%), *Ehrlichia*-like 'Schottii' (2.4%), *Anaplasma phagocytophilum* (1.6%), *Babesia* spp. (EU1) (1.2%), *Babesia divergens* (0.4%) and *Babesia microti* (0.4%) were found. In *I. hexagonus*, *A. phagocytophilum* (5.9%) and *R. helvetica* (0.8%) were detected. In *D. reticulatus* ticks, *Rickettsia* spp. (DnS14/RpA4) (14%) and *Borrelia burgdorferi sensu lato* (0.3%) were detected. The researchers of the article conclude that the spectrum of ticks and tick-borne pathogens (including potential zoonotic pathogens) is much broader than previously thought²⁶.

To invest whether the pet-owning residents of the Netherlands are aware of this threatening risk, the second hypothesis of this study is: Dutch pet owners with knowledge about tick-borne diseases, are more likely to take care of a continuously adequate level of tick protection in their pet than owners with inadequate knowledge about ticks.

PREVENTION OF TICKS AND TICK BITES

For public health and animal health, it is essential to prevent transmission of tick-borne pathogens. There are different ways to prevent ticks and tick-bites, which can be divided in prevention of tick exposure and prevention of attachment^{2,34}.

Environmental prevention is based on the identification of risk areas, such as forest and high grasses. These areas should be avoided especially during the tick season. In the surroundings near the home of dogs/cats, the risk of catching ticks can be reduced by, for example, mowing high grass and bushes and clearing falling leaves on the ground in time^{2,34}.

For individual tick prevention, the control is largely based on the use of acaricides. A variety of different commercial products with repellent and/or acaricidal properties are licensed for both humans and animals. These products are available in various formulations such as shampoos, powders, soaps, sprays, collars, oral administration, pour-on, and spot-on applications^{31,35,36}. In attachment 1 an overview of registered active components used for tick prevention in dogs and cats in the Netherlands is shown (table 1.1 & table 1.2).

It is not clear on which factors pet owner's choices between different tick prevention products are based. Hereby comes that most tick prevention products are freely available. Since registered commercial products for tick prevention are for topical or oral use, this study will invest what kind of tick prevention method is most frequently used for Dutch companion animals.

TICK REMOVAL METHODS

Not all pet owners prevent their pets against ticks. Absence of tick prevention could have different reasons. For example, not all pet owners do have sufficient control over their pets to apply tick prevention measures. Even when a tick prevention method is applied, a dog or cat is still at risk to infestation by ticks. When this occurs, it is essential to remove the tick as soon as possible (e.g. preferably within 16 hours to prevent the transmission of *Borrelia* spp.)²⁴.

Nowadays there are several tools available on the market for the mechanical removal of ticks. For example: tick twisters, tick lasso's, tick removal cards and pen-tweezers (See figure 1.4)⁶⁰⁻⁶³. Forceps are also commonly used for the removal of ticks and some people remove ticks using their fingers. Despite all these available tools, there is not much scientific literature about the effectiveness of tick removal methods. When removing a tick, the aim is complete removal of the tick, including mouthparts and the cement the tick has secreted^{39,40}. If mouthparts retain, inflammatory swelling or granuloma formation can occur^{39,41}. Squeezing into the tick-body should be avoided because otherwise infectious gut contents can enter the wound / attachment site, theoretically this can facilitate transmission of pathogens³⁹⁻⁴¹. Classical removal methods can have different grabbing mechanisms like jaws, a V-shaped slot or a string. These different grabbing mechanisms have the similarity that you have to place them as close to the skin as possible, to ensure the tick can be removed completely with no remnants in the skin. The operating of this tools can be divided into tools that either pull or twist. However, literature is contradictory as to whether pulling or twisting is more effective at removing the tick. The Dutch National Institute of Public health and Environment (RIVM) advise to remove ticks as soon as possible when noticed, with a pointed tweezer. The tweezer should be placed around the head of the tick, as close as possible to the skin, before the tick can be pulled out gently. In several articles twisting is not recommended because of the chance that mouthparts break and retain in the skin^{41,42}. In a study by Stewart (1998) there was no difference found in effectiveness between 3 commercial tick removal tools and normal non-tissue tweezers when removing adult *Dermacentor variabilis* and *Amblyomma americanum* ticks. However, with the removal of nymphs of these tick species, the commercial tools were more successful⁴³. In a more recent study by Duscher (2012) five commercial tick removal methods were tested; pen-tweezers, the Tick Twister[®], "lasso", i. e. Trix[®] tick remover, adson forceps and "card", i. e. TickPic[®]. The conditions of the tick's mouthparts, used forced, time needed for removal and reaction of the dog when using the tick removal methods was analysed. During this research 596 female *I. ricinus* were removed by veterinarians with the 5 commercial tools, and the V-shaped tick twister was most successful based on mouthparts, used force, time needed for removal and reaction of the dog⁴⁰.

Acaricidal products for removal of ticks are also discussed in literature. Some active ingredients block the respiratory system of the tick, and thereby choking the tick. However, due to the low respiration of ticks, this method takes too much time to be effective and therefore there is an risk of pathogenic transmission^{39,40}.



Figure 1.4: Tick removal devices 60-63

MATERIALS AND METHODS

This research project was performed through a survey among Dutch dog/cat owners and with use of the Bayer tickscanner application. With the help of social media, sending emails, visiting veterinary practices and distributing posters in veterinary practices, Dutch dog and cat owners were approached to complete a short online questionnaire. Owners were also asked to submit ticks detected on their pets during the months June, July, August, September and October (2019) through the Bayer tickscanner application for analysis. Both, the survey and submitting ticks, were separate parts of this research project. This means that owners who filled out the survey, did not necessarily need to submit a tick to participate in the research and vice versa. PCR and RLB in an independent laboratory (Utrecht Centre for Tick-borne Diseases) were used to determine the species and screen the ticks on the presence of pathogens. The results of the laboratory were analyzed for this research project. In this study, the aimed minimal sample size was calculated to be minimal 87 ticks, based on a confidence level of 95% and the prevalence of 1.6 % of the pathogen *Anaplasma phagocytophilum* in Dutch *Ixodes ricinus* ticks.

The survey for dog and cat owners was available online starting on the 10th of August 2019 up to and including 30 September 2019. The survey was aimed at gaining more insight into used tick prevention and tick removal methods by pet owners. The surveyed owners were asked whether they used tick preventatives for their pets, which product they use for this and how often they apply the named prevention method. Owners who answered to treat their pets, were also asked to exemplify their reasons for treatment. In addition, all survey participants were asked to name the tick-borne disease(s) that they have knowledge of. Participating owners of this survey do not correspond with the owners which send in ticks through of the Bayer tickscanner application⁵⁶. The questionnaire can be found in attachment 2.

The number of months that an animal is protected, was calculated using product information as licensed and frequency with which the owner applied the product. To perform this calculation, 10 participants were excluded from the analysis which included data regarding 'months protection' because they were not able to mention the name of the product they use. Furthermore only data regarding licensed tick prevention products was used for further statistical analysis. Before the results of the survey could be analyzed, data was checked for normal distributions. When absent, a normal distribution this was created from the obtained data to be able to perform a statistical analysis. Excel and R-studio were used for analysis of the found data. To screen data for significant associations, the χ^2 -test, the Kruskal-Wallis test and the Fisher exact test were applied. To be able to draw conclusions from the results of the survey, associations between 'knowledge about tick borne diseases' & 'application of tick prevention treatment', 'knowledge about tick-borne diseases' & 'months protection', 'knowledge about tick-borne diseases' & 'removal method' & 'location of purchase' and 'knowledge about tick-borne diseases' were examined.

RESULTS

TICK ANALYSIS

With help of the Bayer tickscanner application, 811 ticks were reported in the application in the period from June '19 up and including October '19. Of the reported ticks (811), 700 ticks were actually submitted for analysis, the other 111 weren't submitted. From these submitted ticks, 655 were specified as *I. ricinus* ticks (630 adults, 25 nymphs), 33 as *I. hexagonus* (21 adults, 11 nymphs, 1 larvae), 11 as *D. reticulatus* and 1 as *R. sanguineus* (nymph). Calculated population proportions of the found tick species are shown in table 3.1. Of the owners who submitted a tick through the tickscanner application, 340 owners indicated that the pet on which the tick was found, was treated with tick prevention products.

Of the 700 submitted ticks, 554 ticks were obtained from dogs and 146 ticks from cats. From the ticks found on dogs, 523 were specified as *I. ricinus* (508 adults, 15 nymphs), 19 as *I. hexagonus* (14 adults, 5 nymphs), 11 as *D. reticulatus* (only adults) and 1 *R. sanguineus* nymph. From the submitted ticks found on cats, 132 were identified as *I. ricinus* (122 adults, 10 nymphs) and 14 as *I. hexagonus* (7 adults, 6 nymphs, 1 larvae), see table 3.1.

Table 3.1: Population proportions calculated from submitted ticks

Tick species	Population proportion (n. 700)	Dog (n. 554)	Cat (n. 146)
<i>I. ricinus</i>	93.6%	94.4% (523)	90.4% (132)
<i>I. ricinus</i> adults	90%	91.7% (508)	83.6% (122)
<i>I. ricinus</i> nymphs	3.6%	2.7% (15)	6.8% (10)
<i>I. hexagonus</i>	4.7%	3.4% (19)	9.6% (14)
<i>I. hexagonus</i> adults	3%	2.5% (14)	4.8% (7)
<i>I. hexagonus</i> nymphs	1.6%	90.3% (5)	4.1% (6)
<i>I. hexagonus</i> larvae	0.1%	0% (0)	0.7% (1)
<i>D. reticulatus</i> (adult)	1.6%	2% (11)	0% (0)
<i>R. sanguineus</i> (nymph)	0.1%	0.2% (1)	0% (0)

SURVEY DOG/CAT OWNERS

Through the survey, owners were asked a variety of tick-related questions. The survey was completed by 184 pet owners in the Netherlands. The questions were based on used removal method, used tick-prevention treatment and knowledge about ticks. The complete results of the survey are shown in attachment 2.

Removal methods

Among dog and cat owners, tick twisters (figure 3.1) are most used when removing a tick (55%). No tool was used by 16% of the surveyed owners who remove ticks from their pets with their fingers. Other used removal methods were tweezers, tick cards and others (e.g. tick lasso).

Tick-prevention treatment

The survey showed that 72% of the surveyed pet owners use an anti-tick treatment (spot-on treatment, impregnated collar or oral treatment). For 53,8%, the main reason to treat their pets with anti-tick products is that both the owner and their pets could get infected with a tick-borne disease. Of the participating owners, 34,1% treat their pet because they think only their pet could get a tick-borne disease. A small group of 4,6% of the owners treats their animal because they think ticks are disgusting. Also other reasons were mentioned (7,6%), varying from 'the dogs are itchy' to 'we don't want flees'. Among the pet owners, 28% does not apply any kind of tick prevention to their pets. Figure 3.2 shows points of sale used by pet owners for purchase of tick preventatives. When the participants who said to apply tick prevention were asked which product is used for prevention, 18.2% of the participants was not able to name the name of the product. From the participants who knew which product they used for prevention, Bravecto™ (active component fluralaner) was used most frequently (22,7%). The second mostly used product (12.9%) are electromagnetic ceramic beats. Seresto, a collar with active components flumethrin and imidacloprid, is the third most used product, with 9.1%. Participants who weren't able to name the product they apply for tick prevention, answered that the product used for tick prevention is applied by their veterinarian during their annual health check.

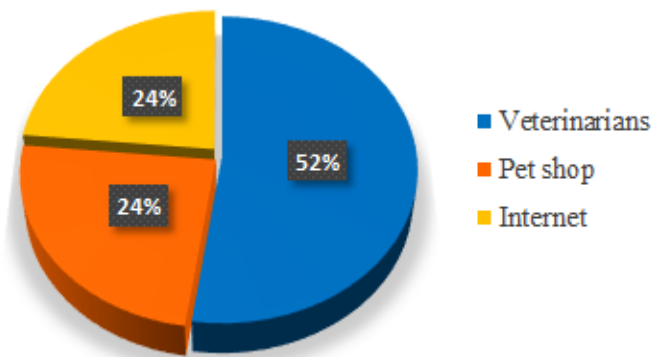


Figure 3.2: Purchase of tick prevention products.

KNOWLEDGE ABOUT TICK-BORNE DISEASES

Knowledge about tick borne diseases was not present in 8.2% of the pet owners, they weren't able to name any tick-borne disease. Among the pet owners 77.1% was able to mention 1 tick-borne disease. A total of 15.1% of the pet owners was able to mention 2 or more tick-borne diseases, and therefore is said to have knowledge about tick-borne diseases. In table 3.2 the percentage of pet owners that knows a certain number of tick-borne diseases, is visible. From tick-borne diseases, Lyme disease is the most frequently mentioned tick-borne disease among owners, 89% is aware of the disease, as shown in table 3.3. Besides Lyme disease, babesiosis is the second most known tick-borne disease pet owners mention (15,8%). Other tick-borne diseases (e.g. ehrlichiosis, anaplasmosis, tick borne-encephalitis) are known by a small group and 8% of the participants is not able to mention any tick-borne disease.

Table 3.2: Number of tick-borne diseases mentioned by pet owners.

Number of known tick-borne diseases	Percentage of pet owners (n=184)
0	8.2%
1	77.1%
2	5.4%
3	6.5%
4	2.7%
5	0.5%

Table 3.3: Known tick-borne diseases mentioned by pet owners.

Tick-borne disease	% of owners
Borreliosis (Lyme disease)	88,6%
Babesiosis	15,8%
Anaplasmosis	12%
Tick-borne encephalitis	4,9%
None	8,2%
Other	5,4%

ASSOCIATIONS

To be able to draw conclusions from the survey, following associations were analyzed: tick-prevention treatment versus knowledge of the owner, location of purchase of the tick-prevention treatment versus knowledge of the owner and location of purchase of the tick-prevention treatment versus months of protection of the animal. The results of the assessment of these associations are shown below.

TICK PREVENTION VS. KNOWLEDGE

The expected relation: when an owner has knowledge (able to mention 2 or more tick-borne disease) about tick-borne diseases, he/she is more likely to apply tick-prevention products. Owners with no knowledge about tick-borne diseases (able to mention 0 or 1 tick-borne disease), are less likely to apply tick prevention treatments to their pets. This association was assessed because a significant difference was expected between whether an owner has knowledge about tick-borne diseases and whether or not an owner treats his or her pet against ticks. The expected relation: when an owner has knowledge about tick-borne diseases, he/she is

more likely to treat their pet against ticks. Before analysis, data was transformed into a normal distribution. Analysis of this data through a Fisher's exact test gives a p-value of 0.34, which suggest there is no significant correlation. In table 3.4 the results of the analysis are represented.

Table 3.4: tick-prevention treatment versus knowledge about tick-borne diseases

Number of known tick-borne diseases by dog/cat owners	% of owners that does apply tick prevention	% of owners that doesn't apply tick prevention
0	9%	10%
1	73%	79%
2	6%	4%
>2	13%	8%

KNOWLEDGE VS MONTHS PROTECTED

This association was assessed because a relation was expected between the knowledge of the owner about tick-borne diseases and the level of protection of their animal against ticks, defined in "months protected per year". The number of protected months per year was calculated with help of the frequency of application and the duration of efficacy of the used tick prevention method according to the product licence. The expected relation: when an owner has knowledge about tick-borne diseases, he/she is more likely to apply sufficient tick-prevention. Dogs and cats of owners with no knowledge about tick-borne diseases (able to mention 0 or 1 tick-borne disease), are usually protected against ticks between 0 and 4 months. Dogs and cats of owners with greater knowledge (able to mention 2 or more tick-borne diseases), were protected 3 to 12 months per year (with an average of 7 months). The results of this association are displayed in a box plot, shown in figure 3.5. Analysis on the data was performed through a non-parametric test (Kruskal-Wallis test), which showed a significant correlation ($p= 0.000083$) between knowledge about tick-borne diseases and the months of protection.

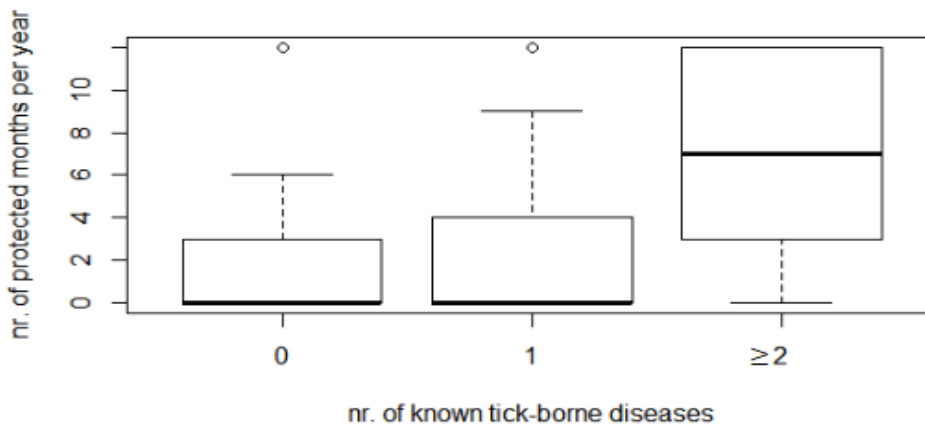


Figure 3.5: Knowledge of the owner (in number of known tick-borne diseases) in relation to the number of months which their pet is protected. Note that 2 tick-borne diseases two or more.

LOCATION OF PURCHASE VS. KNOWLEDGE

This association was assessed because a relation was expected between the point of sale where owners purchase their products for tick-prevention and the level of knowledge of an owner about tick-borne diseases. It is expected that owners who purchase tick-prevention products at their veterinarian, will be better educated than owners who purchase tick-prevention products at other locations (e.g. internet, pet store). Analysis through a χ^2 test gives a p-value of 0.0064. This indicates that owners with knowledge about tick-borne diseases buy their tick prevention treatment significantly more frequently at the veterinarian.

KNOWLEDGE VS. REMOVAL METHOD

This association was assessed because a relation was expected between whether an owner has knowledge about tick borne diseases (able to mention 2 or more tick-borne diseases) or not (able to mention 0 or 1 tick-borne disease) and the used removal method. It is expected that owners who have no knowledge about tick-borne diseases, remove ticks from their pets by hand more often. In table 3.2 the number of known tick-borne diseases and the number of owners using their hands or tick removal device is shown. Analysis of this data through a Fisher's exact test shows this correlation is not significant ($p = 0.5439$). Investigating this association's results (table 3.2), a clinically relevant trend is seen in which knowledge about 2 or more tick-borne diseases, seems to show a relation to using a device for tick removal. Among the owners that have tick-related knowledge, using a device for removal is preferred.

Table 3.2: The number of know tick-borne diseases versus the number of owners using their hands/fingers or a tick removal method when removing a tick from their pet.

	Removal with fingers	Removal with device
No knowledge about tick-borne diseases	29 (16.9%)	115 (66.9%)
Knowledge about tick-borne diseases	3 (1.7%)	25 (14.5%)

LOCATION OF PURCHASE VS. LICENSED OR UNLICENSED PRODUCT

This association was assessed because a relation was expected between whether the location of purchase of the tick-prevention product and whether the product is licensed or not. It is expected that owners who purchase their tick-prevention treatment in a pet shop or online, more frequently buy unlicensed products. Analysis of this data through a Fisher's exact test shows a significant correlation ($p= 2.91e-12$).

DISCUSSION

During the research period, owners were encouraged to submit ticks through the tickscanner application. From the ticks, *I. ricinus* is the most detected tick infesting dogs and cats, with a population proportion of 93.6%. The proportion identified as *I. hexagonus* is 4.7% and for *D. reticulatus* is 1.6%. Among the pet owners who participated in the survey, which did not necessarily submit a tick, 72% of the pet owners uses a kind of tick-prevention. Bravecto™, with active component fluralaner, is the product most used (22,7%) among Dutch pet owners. A biological, clinical trend is seen between knowledge about tick borne diseases and applying treatment against ticks, however, no significant correlation was found. Owners with knowledge about tick-borne diseases, also seem to take care of more months of tick-prevention for their pets than owners without knowledge about tick-borne diseases. Furthermore owners with knowledge about tick-borne diseases are more likely to use a device for tick removal. Furthermore, owners that buy tick prevention treatment at the veterinarian have significantly more knowledge about tick-borne diseases.

Although the method of collection ticks differs between this research and Nijhof (2007)'s research, the proportion of ticks identified as *I. ricinus* (88.9%) found in this research is quite similar to the found population proportion for *I. ricinus* (93.6%) in the research of Nijhof (2007). However, the subdivision between the different lifestadia, differ between this research and Nijhof (2007). Nijhof (2007)'s found prevalences for *I. hexagonus* (7.6%) and *D. reticulatus* (1.7%) are quite similar to the found population proportions *D. reticulatus* (1.6%) in this research. For *I. hexagonus* a difference of 3% is seen between this study and the studies by Nijhof (2007) and Jongejan (2019). A possible explanation for this difference could be different months for collecting ticks. Nijhof (2007) collected ticks over a more longer period compared to this study and Jongejan (2019) collected ticks during the months May and June, whereas this study collected ticks between June until October. The other small differences that are seen could be explained by the fact that ticks analysed in the research of Nijhof (2007) were both collected from the field and submitted, while the ticks obtained for this research were specifically collected from dogs and cats, which could cause a difference in the ratio of tick species found. This research suggest that the population proportions found, do not enormously differ from the prevalence of tick species found between July 2005 and October 2006 in the research of Nijhof (2007), which would indicate that the population proportions of tick species found on cats and dogs in the Netherlands between June 2019 and October 2019, in general, remained quite similar to the prevalences published in 2007. In attachment 1, table 1.3 an overview of the population proportions found by Nijhof (2007), Jongejan (2019) and this research can be found.

For this research all data supplied through the Bayer tickscanner application between June 2019 and October 2019 was used. The fact that this study used the tickscanner application to submit ticks, could create a bias towards individuals that decide to participate versus those that discard the tick. Of the 700 submitted ticks, 554 ticks were obtained from dogs and 146 ticks from cats. A lot more ticks were found on dogs, which could have various reasons. It might sound assumable that ticks are attracted more to dogs, but options in which cat owners don't notice the tick or don't submit the ticks, should be kept in mind. It is possible that cats do remove ticks from themselves while grooming, before its owners even notice the tick. Of the owners who submitted a tick through the tickscanner application, 340 owners (41.9%) indicated that the pet on which the tick was found, was treated with tick prevention products. This is a point of discussion, since this might be an indication that used tick prevention methods don't give enough protection against ticks. Another explanation could be that the used tick prevention products aren't applied in the correct manner. When compared to the survey results of this study, in which 72% of the participants answered to apply tick prevention, the 41.9% is much lower. This difference could be explained by the fact that the questionnaire was spread partly through flyers in veterinary practices, which could create a selection bias among the participants. Owners who got familiar with the questionnaire at a veterinary practice might be more likely to use tick-prevention products.

Since most of the registered commercial products for tick prevention are for topical use, the aim of this study is to investigate what kind of tick prevention method is most frequently used for Dutch companion animals. From the pet owners who filled out the survey, 132 (72%) indicated that their pet was treated with tick prevention products. This is relatively low compared to research performed by Matos (2015) in Portugal, in which 90.2% of the surveyed pet owners indicated to apply tick prevention⁵³. However, among our 72% of the participants that apply tick-prevention products, licensed and non-licensed products are included, which suggests the use of effective products to be even lower. A part (28%) does not apply any kind of tick prevention to their dog or cat. This study did not elaborate further on reasons of owners not to treat their pet. However, this could be an interesting subject for further research. If the reasons not to treat a pet against ticks are better understood, this information can be used to motivate people to treat their pets. From the participants who knew which product they used for prevention (81.8%), Bravecto™ (with the active component fluralaner) was used most frequently (22.7%). This is in agreement with the study among veterinarians linked to this research by D. Spreen, who conducted a survey among veterinarians between June 2019 and September 2019, where the most frequently recommended product for tick prevention, turned out to be Bravecto™. Bravecto™, which is available as a spot-on application and as an oral tablet, contains the long-acting component fluralaner, which ensures that this product only needs to be applied 4 times a year in contrast to 12 times a year for most spot-on products. A study performed among dog owners by Lavan (2017), found that the sustained activity of fluralaner, with its 3 months efficacy, could lead to improved compliance to tick-prevention of the owner. The fact that Bravecto™ (with active component fluralaner) comes out as the most frequently used product among Dutch pet owners, could therefore also lead to improved compliance of tick-prevention treatment. Among pet owners there might be a certain anxiousness for Bravecto™, since several cases of pathologies and deaths of dogs (with various races and ages) coincided with the intake of the product have been reported⁴⁷. News messages like these on social media could discourage pet owners to use Bravecto™ or it might even discourage them to use any kind of tick-prevention treatments because they fear toxic effects. Seresto, a collar with active components flumethrin and imidacloprid, is the third most used product (9.1%), this product is only for sale in veterinary clinics. This result is in agreement with the linked research of D. Spreen, in which Seresto (a collar with 8 months efficacy against ticks) is the third most recommended product (12.5%) by Dutch veterinarians. The corresponding survey outcomes between the survey among veterinarians and the survey among pet owners, again, are a sign of selection bias among the participants. This bias creates an unrepresentative outcome, because pet owners who visit the veterinarian are more likely to apply (licensed) tick prevention.

Notable is that the second most used product for tick prevention are electromagnetic ceramic beads (12.9%), which are not scientifically proven to have a tick repulsive effect. Websites which sell these products, claim that the ceramic beads create a certain resonance to repel ticks. No scientific literature can be found to prove this effect. Furthermore, the survey was spread through social media, which makes it possible that the survey was spread among certain groups of pet owners who might have a specific idea about taking care of pets, which could be the case since ceramic beads (a non-licensed tick-prevention product) are used strikingly frequently by pet owners that filled out the survey. The spread of the survey among social media, and thus certain groups with the same ideals and ambitions, might be the reason for an over-representation of a certain group of pet owners in the survey results. Also, the owners which use unlicensed prevention products, often believe these products are effective. These owners might, for instance, still show knowledge about the risks of tick-borne diseases, which could create a bias regarding other questions. Another reason for using a non-licensed tick-prevention method, could be a fear for toxic effect of tick-prevention treatment. Although Bravecto™, and the other licensed tick-prevention treatments, have proven to be safe⁵¹, owners might still rather use non-licensed methods, which aren't proven to be effective.

The fact that 16% of the participating owners remove ticks from their pets by hand, implies that this 16% of the participants does not care about or is not aware of the risks of tick-borne diseases. On the multiple choice question why pet owners treat their pet against ticks, 53.8% of the participants choose the answer

‘Because me and my pet could get infested with a tick-borne disease’. Since this question was asked as a multiple choice question, it is possible that this answer stood out like the most correct answer for the participants. If this question was formulated as an open question, it could probably have shown another outcome. However, 34.1% of the owners treat their pet because they think only their pet could get a tick-borne disease, which implies that this part of the participants is not aware or does not care about the zoonotic risks of tick-borne diseases. A small group of 4,6% of the owners treats their animal because they think ticks are disgusting, this implies that this small share of owners don’t think tick-borne diseases are a threat to them or to their pet. Also other reasons were mentioned (7,6%), varying from ‘the dogs are itchy’ to ‘we don’t want fleas’, which implies that this part of the owners is not aware either of the risks of tick-borne diseases or is confused about the difference between fleas and ticks. It is also possible that pet owners value flea protection more than tick prevention and use products which protect against both fleas and ticks.

Of the survey participants, 18.2% was not able to name the product they apply for tick prevention. In the remarks field of the questionnaire some participants who couldn’t name the product, answered that the product used for tick prevention is applied by their veterinarian during their annual health check. However, most licensed products have to be applied 4 to 12 times a year, to give a year-round, sufficient effect. When a pet doesn’t have health problems, it is not common to visit the veterinarian this often, so it is assumable that the pets of these owners are not sufficiently protected against ticks during the year. Another option is that the participants are confused with other products used for parasite prevention (e.g. worm tablets, anti-flea agents), which in this case also wouldn’t be applicated often enough. This would be in line with the comments that were written to answer the question why owners apply tick prevention (e.g. ‘the dogs are itching’ and ‘we don’t want flees’). It would be an educational opportunity for the veterinarian to inform about different kinds of ectoparasites and, even more important, sufficient protection against the various kinds of ectoparasites. Research performed by Beck (2013) showed that even when owners apply tick prevention, the method is often (56%) not used as recommended on the label. Additionally a survey concerning ticks and tick-prevention methods among pet owners was conducted in the area of Berlin, which concludes that prophylactic and/or therapeutic measures to prevent tick-infestation are not performed correctly in the majority of the cases. This was also concluded in research of Matos (2015). This is in agreement with research performed by Leschnik (2013), which concluded that more educational training for dog owners is necessary to make the application of tick-prevention products effective, regarding the prevention of tick-borne diseases. A veterinary recommendation requires that the veterinarian clearly provides a comprehensible and distinct advice for the pet owner.

In this research ‘having knowledge about ticks’ is linked to one parameter, namely being able to name 2 or more tick-borne diseases. In research performed by Niesobecki (2019), a perceived prevalence and (self-rated) knowledge about Lyme disease by inhabitants of Connecticut and Maryland (USA) was significantly associated with pet tick prevention⁵². In a study on public perceptions regarding Lyme disease performed by Beaujean (2013) in the Netherlands, 95% of the participants perceives Lyme disease as severe to very severe⁵⁴, which shows that a high percentage of Dutch inhabitants is familiar with the severity of Lyme disease. When investigating this, Beaujean (2013) reported that only 35% of the people showed a good general knowledge of Lyme disease⁵⁴, which indicates that being familiar with Lyme disease doesn’t necessarily indicate a good general knowledge about tick-borne diseases. Since most participants in this research (88%) are familiar with Lyme disease, the discriminant for having knowledge about tick-borne diseases was established at knowing at least 2 tick-borne diseases. When participants know 2 or more tick-borne diseases, it is assumable that knowledge is present.

In the relation between the knowledge of pet owners and the number of months their pet is protected against ticks, a significant relation was seen in which pet owners that have knowledge about tick-borne diseases, seem to take care of more months of tick-prevention for their pet (3-12 months). However, in the linked research of D. Spreen (2019), ticks in veterinary practices were seen from March until October (8 months), with a peak season from May up until August. When these months are taken into account, a part of the owners with knowledge about tick-borne diseases still does not apply enough tick prevention treatments.

CONCLUSION

In this research, *I. ricinus* is the most detected tick infesting dogs and cats, with a population proportion of 93.6%. The found research proportion for *I. hexagonus* is 4.7% and for *D. reticulatus* is 1.6%.

Among the pet owners who participated in this research, 72% of the pet owners uses an anti-tick method (spot-on treatment, impregnated collar or oral treatment). Bravecto™, a spot-on and chewable tablet with active component fluralaner, is the most used treatment (22,7%) among Dutch pet owners.

In this research an estimation of a pet owner's knowledge was made depending on the number of known tick-borne diseases. Since Lyme disease is commonly known among the participants (88%), the decision was made to assign the label 'having knowledge of tick-borne diseases' to participants able to mention 2 or more tick borne diseases (15.1%). According to this research, pet owners with knowledge about tick-borne diseases, seem to treat their pet 13% more often than pet owners with less knowledge about tick-borne diseases. Although definite conclusions cannot be drawn, from the biological, clinical trend that is seen, a conclusion could be that owners with no knowledge about tick-borne diseases have little motivation to use treatment for tick prevention for their dog or cat. Owners with better knowledge about tick-borne diseases, also seem to take care of a continuously efficient level of tick-prevention for their pet and are more likely to use a device for tick removal. Furthermore, it can be concluded that owners which buy tick prevention treatment at the veterinarian have significantly more knowledge about tick-borne diseases.

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ATTACHMENTS

ATTACHMENT 1

Table 1.1: Registered active components for tick prevention for dogs in the Netherlands ³⁷.

Active component	Way of administration	Duration of effectivity	Minimal age dog	Exposure
Carbamates & organophosphates				By skin contact
Carbamates	Collar, powder, aerosol, spot-on	2-4 months	3 months	
Organophosphates	Collar, powder, aerosol, spot-on	4-5 months	3 months	
Chloronicotiny / nicotinoids				By skin contact
Dinotefuran / Pyriproxyfen / Perm.	Spot-on	3-4 weeks	7 weeks / 1.5 kg	
Imidacloprid / Flumethrin	Spot-on	7 - 8 months	7 weeks	
Imidacloprid / permethrin	Spot-on	3 -4 weeks	7 weeks / 1.5 kg	
Phenylpyrazoles				
Fipronil	Spray	4 weeks	2 days	
Fipronil	Spot-on	4 weeks	8 weeks / 2 kg	
Fipronil / Methoprene	Spot-on	4 weeks	8 weeks / 2 kg	
Fipronil / Permethrin	Spot-on	4 weeks	8 weeks / 2 kg	
Fipronil / Pyriproxyfen	Spot-on	2 - 4 weeks	10 weeks / 2 kg	
Fipronil /	Spray	4 weeks	2 days	

Pyriproxyfen				
Pyriprole	Spot-on	4 weeks	8 weeks / >2kg	
Isoxazolines				By blood feeding
Afoxolaner	Tablet	4 weeks	8 weeks / 2 kg	
Afoxolaner / Milbemycin	Tablet	4 weeks	8 weeks / 2 kg	
Fluralaner	Tablet / spot - on	12 weeks	8 weeks / 2 kg	
Lotilaner	Tablet	4 weeks	8 weeks / 1.3 kg	
Sarolaner	Tablet	5 weeks	8 weeks / 1.3 kg	
Oxadiazins				By skin contact
Indoxacarb / Permethrin	Spot-on	5 weeks	8 weeks / 1.2 kg	
Pyrethrins & pyrethroids				By skin contact
Deltamethrin	Collar	6 months	7 weeks / 1.5 kg	
Flumethrin / Propoxur	Collar	6 months		
Permethrin	Shampoo / Powder	2 - 4 weeks	3 months	
Permethrin	Spot-on	4 weeks	2 weeks	

Table 1.2: Registered active components for tick prevention for cats in the Netherlands ³⁸.

Active component	Way of administration	Duration of effectivity	Minimal age cat
Carbamates & organophosphates			
Carbamates	Collar, spot-on, powder	2-4 months	3 months
Organophosphates	Collar, spot-on, powder	4-5 months	3 months
Chloronicotiny / nicotinoids			
Imidacloprid/flumethrin	Collar	7-8 months	10 weeks

Phenylpyrazole			
Fipronil	Spot-on	4 weeks	8 weeks
Fipronil / Methoprene	Spot-on	2 weeks	8 weeks / 1 kg
Fipronil / Methoprene / Eprinomectin / Praziquantel	Spot-on	3 weeks	7 weeks / 0.6 kg
Fipronil / Pyriproxyfen	Spot-on	1 week	10 weeks / 1kg
Fipronil / Pyriproxyfen	Spray	4 weeks	2 days
Isoxazolines			
Fluralaner	Spot-on	12 weeks	11 weeks / 1.2 kg
Fluralaner / Moxidectin	Spot-on	12 weeks	11 weeks / 1.2 kg
Lotilaner	Tablet (oral)	4 weeks	8 weeks / 0.5 kg
Sarolaner / Selamectin	Spot-on	4-5 weeks	8 weeks / 1.25 kg

Table 1.3: Population proportions found in Nijhof (2007), Jongejan (2019) and this research.^{26,65}

	Population proportions Nijhof (2007) (n = 4298, July 2005 – October 2006)	Population proportions Jongejan (2019) (n = 1005, May + June 2018)	Population proportions current research (n = 700, June – October 2019)
<i>I. ricinus</i> Schapenteek	88.9%	90.0%	93.6%
<i>I. hexagonus</i> Egelteek	7.6%	7.3%	4.7%
<i>D. reliculatus</i> Vlekkenteek	1.7%	2.4%	1.6%
<i>R. sanguineus</i> Hondenteek	0.27%	0.1%	0.1%

135	Tekentang	hulpmiddel	nee						0	0	0	<6		Lyme	1
136	Tekentang	hulpmiddel	nee						0	0	0	<6		.	0
137	Pincet / tekenpincet	hulpmiddel	ja	Advantix	reg	Internet	overig	Spot on (pipet)	12 keer	>4	12	26	Mijn hond/kat kan ziek worden	Borreliose, erlichiose, babesiose	3
138	Tekentang	hulpmiddel	nee							0	0	<6		Lyme, borellia	1
139	Tekentang	hulpmiddel	nee							0	0	<6		geen	0
140	Tekentang	hulpmiddel	nee							0	0	<6		Lyme	1
141	Tekentang	hulpmiddel	ja	EM keramiek	nreg	Internet	overig	halsband	1	1	0	<6	Zowel ik als mijn hond/kat kun	Lyme	1
142	Met de hand/vingers	hand/vingers	nee							0	0	<6		Lyme	1
143	Met de hand/vingers	hand/vingers	ja	Seresto	reg	Dierenarts	dierenarts	Halsband	1	1	7	26	Zowel ik als mijn hond/kat kun	Lyme; Babesiose; Ehrlichiose; Anap	4
144	Met de hand/vingers	hand/vingers	ja	Seresto	reg	Internet	overig	Halsband	1x werkt 6-8 mnd	1	7	26	Zowel ik als mijn hond/kat kun	lyme	1
145	Tekentang	hulpmiddel	nee							0	0	<6		lym	1
146	Tekenharkje	hulpmiddel	nee							0	0	<6		Lyme in Nederland	1
147	Tekentang	hulpmiddel	nee							0	0	<6		Ziekte van Lyme, Ehrlichiose en Bab	3
148	Tekentang	hulpmiddel	nee							0	0	<6		Lyme, borrenogwat	1
149	Tekentang	hulpmiddel	nee							0	0	<6		Lyme	1
150	Tekentang	hulpmiddel	ja	Simparica	reg	Dierenarts	dierenarts	Tablet	12	>4	12	26	Mijn hond/kat kan ziek worden	Lyme	1
151	Tekentang	hulpmiddel	nee							0	0	<6		Lyme	1
152	Tekentang	hulpmiddel	nee							0	0	<6		Lyme	1
153	Tekentang	hulpmiddel	ja	Knoflook bonbons	nreg	Internet	overig	Tablet	elke dag	>4	0	<6	Mijn hond/kat kan ziek worden	.	0
154	Tekentang	hulpmiddel	ja	Bravecto	reg	Dierenarts	dierenarts	Spot on (pipet)	2x	2	6	26	Mijn hond/kat kan ziek worden	Zirke van Lyme, tekenencefalitis, E	3
155	Met de hand/vingers	hand/vingers	nee							0	0	<6	Zowe ik als mijn hond/kat kun	Borreli . Anaplasma; Rickettsia; Ba	>4
156	Tekentang	hulpmiddel	ja	Bravecto	reg	Dierenarts	dierenarts	Tablet	4	4	12	26	Zowel ik als mijn hond/kat kun	Lyme en aanverwant, zenuw stelse	1
157	Tekentang	hulpmiddel	ja	EM kralen	nreg	Internet	overig	Halsband	1	1	0	<6	Mijn hond/kat kan ziek worden	Lyme	1
158	Tekentang	hulpmiddel	ja	Defendog	reg	Dierenarts	dierenarts	Spray	2x	2	2	<6	Antwoord 2 en 3	Lyme	1
159	Met de hand/vingers	hand/vingers	ja	Bravecto	reg	Dierenarts	dierenarts	Tablet	1x	1	3	<6	Mijn hond/kat kan ziek worden	Lyme	1
160	Pincet / tekenpincet	hulpmiddel	ja	Geen idee		Internet	overig	Spot on (pipet)	2	2	2	<6	Zowel ik als mijn hond/kat kun	Ziekte van lyme	1
161	Pincet / tekenpincet	hulpmiddel	nee							0	0	<6		Lime	1
162	Tekentang	hulpmiddel	ja	Stop	nreg	Internet	overig	Spot on (pipet)	12	>4	0	<6	Mijn hond/kat kan ziek worden	Ziekte van Lyme	1
163	Tekentang	hulpmiddel	ja	Adventix	reg	Dierenarts	overig	Spot on (pipet)	6	>4	6	26	Mijn hond/kat kan ziek worden	Lyme	1
164	Met de hand/vingers	hand/vingers	ja	Practic	reg	Dierenarts	dierenarts	Spot on (pipet)	2-3 keer	2	2	<6	Mijn hond/kat kan ziek worden	Lyme	1
165	naar de dierenarts!		ja	Comfortis	reg	Dierenarts	dierenarts	Tablet	6 keer	>4	0	<6	Zowel ik als mijn hond/kat kun	Lime (en dat is al erg genoeg)	1
166	Tekentang	hulpmiddel	ja	Vlooiën en teken	reg	Dierenarts	dierenarts	Halsband	2x	2	12	26	Zowel ik als mijn hond/kat kun	Lyme	1
167	Met de hand/vingers	hand/vingers	nee							0	0	<6		Lyme; Plaatselijke ontsteking van d	1
168	Met de hand/vingers	hand/vingers	ja	Bravecto	reg	Dierenarts	dierenarts	Tablet	4 keer	4	12	26	Zowel ik als mijn hond/kat kun	Alle ben paraveterinair	0
169	Tekentang	hulpmiddel	ja	Frontline	reg	Dierenarts	dierenarts	Spot on (pipet)	4 tot 5 keer	4	4	<6	Mijn hond/kat kan ziek worden	Lyme	1
170	Tekentang	hulpmiddel	ja	geen idee hoe het heet		Dierenarts	dierenarts	Tablet	4	4	*	<6	Zowel ik als mijn hond/kat kun	Lyme	1
171	Tekentang	hulpmiddel	ja	Bravecto of Simpa	reg	Dierenarts	dierenarts	Tablet	1 à 2 x per jaar	1	1,25	<6	Zowel ik als mijn hond/kat kun	Ziekte van Lyme, Babesiose en Ana	3
172	Pincet / tekenpincet	hulpmiddel	ja	Scalibor en strong	reg	Dierenarts	dierenarts	Halsband	Kat 6 x	>4	12	<6	Zowel ik als mijn hond/kat kun	Lyme	1
173	Tekentang	hulpmiddel	nee							0	0	<6		Babesioses; huidirritatie; hotspot d	1
174	Tekentang	hulpmiddel	ja	Bravecto spot on	reg	Dierenarts	dierenarts	Spot on (pipet)	4x	4	12	26	Zowel ik als mijn hond/kat kun	Lyme, babesia, ehrlichia, leish m ani	3
175	Tekentang	hulpmiddel	ja	Stronghold plus	reg	Dierenarts	dierenarts	Spot on (pipet)	3	3	3	<6	Zowel ik als mijn hond/kat kun	Ziekte van Lyme	1
176	Lasso	hulpmiddel	ja	Frontline + middel	reg	Dierenarts	dierenarts	Spray	Hele jaar ongeveer	>4	0	<6	Zowel ik als mijn hond/kat kun	Tig. Maar aan Lyme een hond verlo	1
177	Met de hand/vingers	hand/vingers	ja	Advantix	reg	Dierenarts	dierenarts	Spot on (pipet)	9	>4	9	26	Zowel ik als mijn hond/kat kun	Ziekte van lym	1
178	Tekentang	hulpmiddel	nee							0	0	<6		In Nederland ziekte van Lyme.	1
179	Eerst alcohol er op. Dan	hand/vingers	ja	Geen idee uit mijn	reg	Dierenarts	dierenarts	Tablet	2	2	*	<6	Al het bovenstaande.	Lyme vnl. Maar allergie zal ook een	1
180	Tekentang	hulpmiddel	ja	Van de dierenarts	reg	Dierenarts	dierenarts	Spot on (pipet)	1 keer	1	1	<6	Zowel ik als mijn hond/kat kun	Lyme	1
181	Met de hand/vingers	hand/vingers	ja	Bravecto	reg	Dierenarts	dierenarts	Spot on (pipet)	2	2	6	26	Zowel ik als mijn hond/kat kun	Babesiose; Korts	1
182	Tekentang	hulpmiddel	nee							0	0	<6		Ziekte van Lyme, Ehrlichiose, Babes	3
183	Met de hand/vingers	hand/vingers	ja	Bravecto	reg	Dierenarts	dierenarts	Tablet	3	3	9	26	Mijn hond/kat kan ziek worden	Lyme	1
184	Tekentang	hulpmiddel	ja	EMkeramiek	nreg	Internet	overig	Halsband	elke dag	1	0	<6	Mijn hond/kat kan ziek worden	weet de naam niet sorry	0