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# Efficacy of an Imidacloprid 10%/ Flumethrin 4.5% Collar (Seresto®, Bayer) for Preventing the Transmission of *Cytauxzoon felis* to Domestic Cats by *Amblyomma americanum*

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## Abstract

Infection of *Cytauxzoon felis* in domestic cats produces a severe disease characterised by fever, lethargy, inappetence, anorexia, depression, dehydration, icterus and often death. Transmission of *C. felis* to cats is dependent on being fed upon by infected *Amblyomma americanum* (lone star ticks). The purpose of the present study was to determine if application of a 10% imidacloprid/4.5% flumethrin collar (Seresto®, Bayer) on cats prevents transmission of *C. felis* by repelling ticks. Twenty cats were randomised to either a treated (n = 10) or non-treated control group (n = 10) based on their susceptibility to ticks. Cats of high, medium and low tick susceptibility were represented in both groups. Treated cats were fitted with 10% imidacloprid/4.5% flumethrin collars on study day 0 and both groups were then infested with *C. felis*-infected *A. americanum* on study day 30. Tick thumb counts were performed at 24 and 48 hours post infestation. Transmission of *C. felis* was determined by examining blood of cats by DNA extraction followed by PCR amplification with piroplasm-specific primers. Ticks did not attach to any of the 10% imidacloprid/4.5% flumethrin-treated cats. However, ticks attached and fed on all the non-treated control cats. The geometric mean number of ticks attached to the non-treated control cats at 24 and 48 hours was 15.3 and 14.2, respectively. *Cytauxzoon felis* was transmitted to

9 of 10 (90%) non-treated control cats; *C. felis* was not transmitted to any of the treated cats. Transmission of *C. felis* to the non-treated cats was first detected between 8 and 16 days post infestation. Our results indicate that application of the 10% imidacloprid/4.5% flumethrin collar to cats prevented ticks from attaching, feeding and transmitting *C. felis*.

## Introduction

Cats infected with *Cytauxzoon felis* were first reported in southwestern Missouri (Wagner 1976). Since then *C. felis* has been documented in domestic cats throughout the South-central and southeastern United States (Meinkoth and Kocan 2005; Cohn and Birkenheuer 2012). Infection with *C. felis* in domestic cats produces a severe, acute, rapidly progressive disease characterised by fever, inappetance, anorexia, listlessness, dyspnea, icterus, and often death (Wagner 1976; Wagner et al. 1980). Transmission of *C. felis* to cats is dependent on being bitten by an infected tick vector. Two tick species, *Dermacentor variabilis* (Blouin et al. 1984; Blouin et al. 1987), American dog tick, and *Amblyomma americanum* (Reichard et al. 2010; Reichard et al. 2009), lone star tick, have been implicated as vectors of *C. felis* to cats. Repeated transmission trials comparing the abilities of both ticks to transmit *C. felis* to immunocompetent domestic cats have demonstrated that *A. americanum* is a more reliable vector than *D. variabilis* (Reichard et al. 2010). Additionally, natural infections of *C. felis* in wild-caught, unengorged *A. americanum* adults and nymphs have been reported whereas natural infections of *C. felis* in *D. variabilis* were not discovered (Reichard et al. 2010).

A vaccine to prevent cytauxzoonosis has not been developed (Cohn and Birkenheuer 2012). Controlling the transmission of *C. felis* to domestic cats is focused on limiting cat exposure to ticks by strict avoidance of infested environments and application of approved acaricides to cats to prevent tick bites (Meinkoth and Kocan 2005; Cohn and Birkenheuer 2012). The purpose of the present study was to determine if application of a 10% imidacloprid/4.5% flumethrin collar (Seresto®, Bayer) on cats could prevent transmission of *C. felis* by repelling *A. americanum*, as evidenced by lack of tick attachment.

## Materials and methods

### Experimental design

This was a controlled, completely randomised efficacy study. Twenty principal cats were randomised to either a treated (n = 10) or non-treated (control) group (n = 10) based on their susceptibility to ticks. Treated cats were fitted with 10% imidacloprid/4.5% flumethrin collars on study day 0 and then both groups were infested with *C. felis*-infected *A. americanum* on study day 30. Efficacy of the collar was measured by comparing total body thumb counts of attached ticks at 24 and 48 hours post infestation (study days 31 and 32) on both groups of cats. Transmission of *C. felis* was determined by testing blood for infection by PCR with piroplasm-specific primers and microscopy at timed intervals post infestation with *C. felis*-infected *A. americanum*.

### Cats

Experiments utilising cats were governed by an Animal Care and Use Protocol approved by the Oklahoma State University (OSU) Institutional Animal Care and Use Committee. Cats were housed in sealed, climate-controlled isolation rooms in the Animal Resources unit at the Center for Veterinary Health Science at OSU (Stillwater, OK). Food and water was provided *ad libitum*.

Twenty-one cats were used in the study. One cat, the *C. felis* donor cat, was a cytauxzoonosis survivor from a previous transmission trial (Reichard et al. 2009) and the other 20 were principal cats used to evaluate the efficacy of the collar. The 20 principals

were domestic short-haired cats purchased from a commercial supplier (Liberty Research, Inc., USA). All 20 principal cats were intact females, approximately 6 months of age and vaccinated against feline rhinotracheitis virus, calicivirus, panleukopenia virus and rabies virus. The *C. felis* naive status of the principal cats was verified prior to entering study and prior to the efficacy trial by nested PCR amplification using piroplasm-specific primer (described below) of DNA extracted from whole blood.

### Susceptibility of principal cats to *Amblyomma americanum*

To determine their susceptibility to ticks, principal cats were infested with *A. americanum* adults not infected with *C. felis* purchased from the OSU Tick Rearing Facility (Stillwater, OK) on study days –10 or –8. To infest cats, they were anaesthetised via intramuscular injection with 8.0 mg/kg tiletamine and zolazepam (Telazol; Fort Dodge, USA) and placed individually in a wire cage (76.2 x 53.3 x 70 cm) that had a solid bottom. Each cage was placed over a plastic trap pan (104.1 x 73.6 x 12.7 cm) that acted as a catch basin and contained ticks when they fell off the cat. Margins of the trap pans were lined with double-sided tape to prevent ticks from escaping. Twenty-five pairs (i.e. 25 female and 25 male ticks) were placed between the shoulder blades of anaesthetised cats and were allowed to attach and feed at will. Total body thumb counts for attached ticks were performed 48 hours post infestation. To perform thumb counts, cats were again anaesthetised and the entire hair coat of each cat was systematically examined by parting the hairs for attached or unattached ticks. The number of ticks attached to cats were ranked from highest to lowest and then cats were randomly allocated to either a treated (n = 10) or non-treated control group (n = 10). Cats of high, medium and low tick susceptibility were represented in both groups. Ticks were removed after the 48-hour tick susceptibility thumb count.

### Acquisition feeding of *Amblyomma americanum* nymphs on the *Cytauxzoon felis* donor cat

*Amblyomma americanum* nymphs were purchased from the OSU Tick Rearing Facility (Stillwater, OK) and acquisition fed on the *C. felis* donor cat. The donor cat was persistently and subclinically infected with *C. felis*. The donor cat was infested with *A. americanum* nymphs as described above except the thorax and abdomen of the cat was shaved and fitted with a Surgi-Sox™ (DogLeggs, USA) to facilitate tick attachment and minimise tick removal through host grooming. Engorged nymphs were collected daily, placed into paper cartons and stored in a humidity chamber (90 to 98% humidity at 25 °C with a 14-h light-dark photophase) for molting to adults. The parasitaemia of *C. felis* in the donor cat red blood cells during acquisition feeding of *A. americanum* nymphs ranged from 0.003% at time of infestation to 0.002% after the last replete nymph was recovered.

### Efficacy of the 10% imidacloprid/4.5% flumethrin collar

Cats allocated to the treatment group were fitted with the 10% imidacloprid/4.5% flumethrin collar according to the label directions on study day 0. On study day 30, cats in both groups were infested with 25 pairs of *A. americanum* adults that were acquisition fed as nymphs on the *C. felis* donor cat. Infestations were conducted as described above for estimating the principals' susceptibility to ticks. That is, the hair coats of the principal cats were not clipped nor were Surgi-Sox™ used to confine ticks during this part of the study. Total body thumb counts were performed on cats in both groups at 24 and 48 hours post infestation (i.e. study days 31 and 32). Efficacy of the collar against *A. americanum* on cats was calculated by:

$$\text{Efficiacy (\%)} = 100 \times (m_c - m_t) / m_c$$

$m_c$ = geometric mean of live *A. americanum* attached on cats in the non-treated control group

$m_t$ = geometric mean of live *A. americanum* attached on cats in the treated group

### Determination of *Cytauxzoon felis* infection in cats and ticks

Cats were observed daily and given routine physical examinations throughout the study. Blood was collected from cats and tested for *C. felis* infection on approximate study days –10, 30, 38, and continued roughly every other day until infection was confirmed or study day 60 was reached (i.e. 9 days beyond the 21-day maximum prepatent period). Blood was collected via standard, aseptic venipuncture of jugular or cephalic veins into an evacuated tube containing $K_2$-EDTA (Becton Dickinson, USA). Infection was determined by looking for piroplasms in erythrocytes on Wright-Giemsa stained thin blood smears or PCR amplification of *C. felis* DNA extracted from cat blood. DNA was extracted from 200 µl of whole blood using GE Healthcare illustra™ blood genomicPrep Mini Spin Kit (Buckinghamshire, UK) according to the manufacturer's instructions. Cat blood was tested for infection with *C. felis* using a nested PCR and piroplasm-specific primers according to previously published (Bondy et al. 2005; Reichard et al. 2010) protocols. Blood from the *C. felis* donor cat was used as a positive control for DNA extractions and PCR amplifications. Purified PCR water was used for negative control samples.

*Amblyomma americanum* used for the susceptibility and efficacy studies were tested for the absence or presence, respectively, of *C. felis* to verify infection status of the protozoan parasite in the ticks. Ticks were mechanically disrupted by dissection and genomic DNA was extracted using a previously described phenol/chloroform method

**Table 1** Susceptibility of principal cats to infestation with *Amblyomma americanum* adults and allocation to treatment group

| Cat | Ticks attached during susceptibility study | Group allocation |
|---|---|---|
| NRB5 | 1 | Non-treated control |
| ZAK3 | 4 | Non-treated control |
| OIF5 | 6 | Non-treated control |
| ZAI4 | 7 | Non-treated control |
| HIG6 | 8 | Non-treated control |
| GRI4 | 9 | Non-treated control |
| ZAM3 | 12 | Non-treated control |
| GRG2 | 12 | Non-treated control |
| NRH1 | 14 | Non-treated control |
| GRF6 | 17 | Non-treated control |
| GRJ2 | 1 | Collar-treated |
| OIM3 | 2 | Collar-treated |
| NRN3 | 3 | Collar-treated |
| ZAN2 | 5 | Collar-treated |
| OII2 | 6 | Collar-treated |
| ZAI2 | 8 | Collar-treated |
| NRI5 | 9 | Collar-treated |
| OHM4 | 12 | Collar-treated |
| GRC5 | 14 | Collar-treated |
| GRE5 | 16 | Collar treated |

**Table 2** Efficacy of 10 % imidacloprid/4.5 % flumethrin collar applied on study day 0 against *Amblyomma americanum* on cats

| Study day | Geometric mean number of attached ticks | | Efficacy (%) | p value |
|---|---|---|---|---|
| | Non-treated control cats | Collar treated cats | | |
| 31 | 15.3 | 0 | 100 | p < 0.001 |
| 32 | 14.2 | 0 | 100 | p < 0.001 |

(Halos et al. 2004). DNA extracts from ticks were diluted 1:1,000 in PCR-grade water and tested for *C. felis* using the aforementioned nested PCR. To confirm that PCR inhibitors that would generate false-negative results were not present in the extracted DNA from ticks, all these samples underwent an additional PCR amplification utilising general primers and protocols described by Bondy et al. (2005) that amplify piroplasm, tick, or mammalian DNA.

### Necropsy

To verify infection status as determined by PCR analyses, *C. felis*-infected principal cats were euthanised at first confirmation of transmission via intravenous administration of sodium pentobarbitol euthanasia solution and necropsies were performed following standard protocols. All major internal organs were immersion fixed in 10 % neutral buffered formalin for 72 hours, routinely processed through graded alcohols and xylene, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin.

### Statistics

The number of ticks attached at 24 and 48 hours post infestation during the efficacy trial was compared using a Wilcoxon's Rank Sum Test (Sokal and Rohlf 1997). The proportion of cats infected with *C. felis* each day between the collar-treated and non-treated control cats were also compared using a Wilcoxon's Rank Sum Test (Sokal and Rohlf 1997). An alpha value of 0.05 was assumed and statistical computations were made using SAS® 9.2 Macro Language (SAS Institute Inc, USA).

## Results

### Tick susceptibility

All principal cats were susceptible to infestation with *A. americanum* (Table 1). From 25 pairs of *C. felis*-uninfected adult *A. americanum* placed on the cats, at least one adult tick was alive and attached 48 hours after being placed on the cats. The maximum number of ticks attached to a cat during the susceptibility trial was 17. Cats of high, medium and low tick susceptibility were represented in both the treated and non-treated control groups (Table 1).

### Efficacy of the 10 % imidacloprid/ 4.5 % flumethrin collar

Ticks did not attach to cats treated with the 10 % imidacloprid/4.5 % flumethrin collar (Fig. 1 and Table 2). Significantly more ticks were attached at 24 ($p < 0.001$) and 48 ($p < 0.001$) hours post infestation on the non-treated control cats. The geometric mean number of ticks attached to non-treated controls was 15.3 and 14.2, respectively (Table 2). Because ticks did not attach to treated cats, the efficacy of the collar was 100 % on study days 31 and 32.

### Transmission of *Cytauxzoon felis*

As determined by PCR analyses, cats treated with the 10 % imidacloprid/4.5 % flumethrin collar did not become infected (0 of 10 = 0 %) with *C. felis*. Whereas 9 of 10 (90 %) non-treated control cats became infected with *C. felis* during the study (Table 3). One non-treated control cat (i.e. OIF5) did not become infected with *C. felis*. Significantly more ($p < 0.02$) non-treated control cats became infected

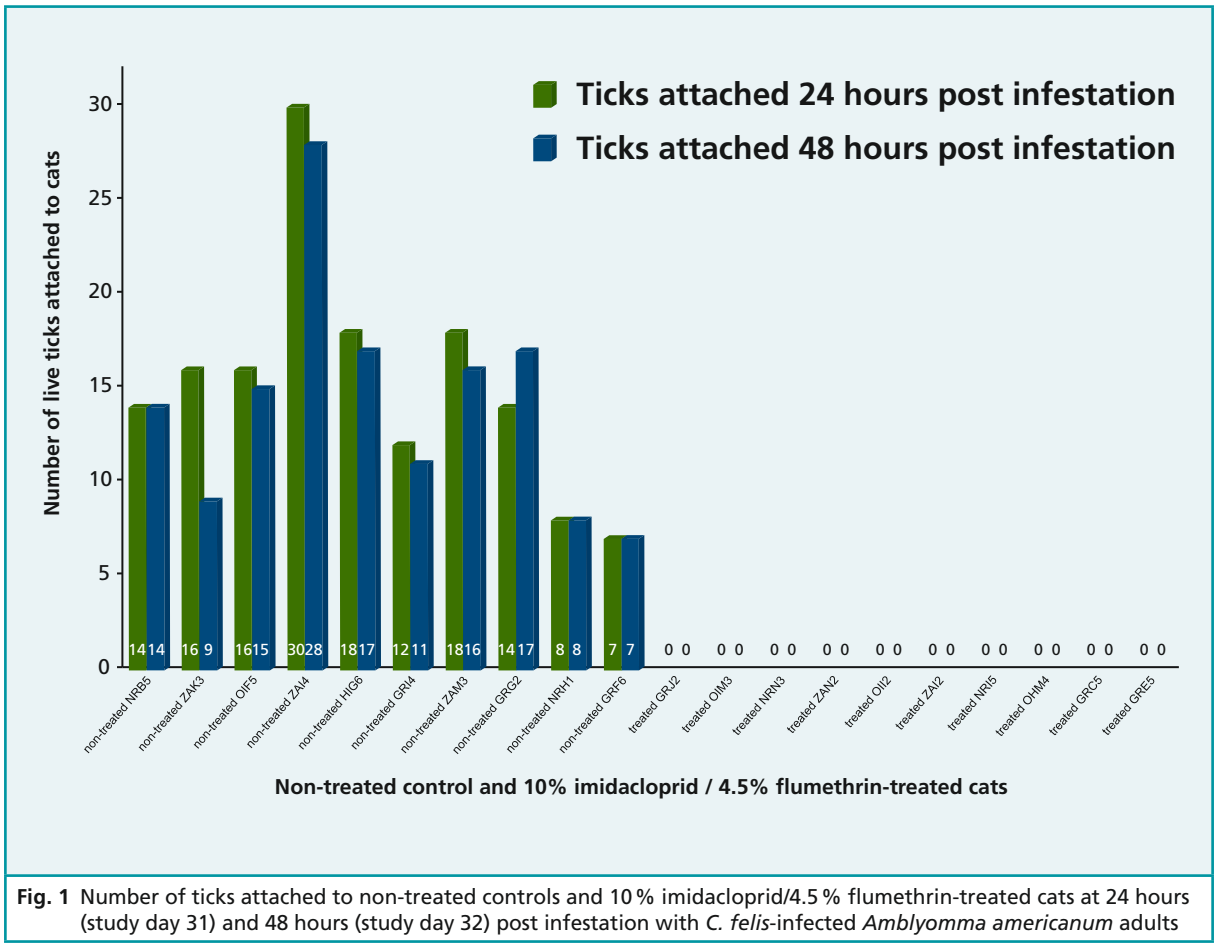


**Fig. 1** Number of ticks attached to non-treated controls and 10 % imidacloprid/4.5 % flumethrin-treated cats at 24 hours (study day 31) and 48 hours (study day 32) post infestation with *C. felis*-infected *Amblyomma americanum* adults

with *C. felis* than those wearing the collar. The prepatent period of *C. felis* in non-treated control cats ranged from 8 to 16 days post infection (d.p.i.). Microscopic examination of stained thin blood films from cats did not reveal *C. felis* piroplasms. Because cats were euthanised at first detection of *C. felis* infection, clinical signs of cytauxzoonosis were not observed in any of the cats.

### *Cytauxzoon felis* infection in *Amblyomma americanum*

A subsample of 28 ticks used to determine the susceptibility of principal cats to infestation with *A. americanum* were reserved and tested for *C. felis* infection. Infection of *C. felis* was not detected in any of these 28 (0 %) *A. americanum*. A subsample of 72 adult *A. americanum* that had been acquisition fed as nymphs on the *C. felis* donor cat and utilised to determine the efficacy of the collar were tested for *C. felis* infection. Three of these 72 (4 %) were infected with *C. felis*.

### Necropsy of *Cytauxzoon felis*-infected cats

Necropsy findings in all 9 cats euthanised at first detection of *C. felis* infection were consistent with early cytauxzoonosis. Results included mild pneumonia, mild-to-moderate lymphadenopathy and mild anemia. Impression smears of lung and spleen confirmed presence of *C. felis* schizonts. Histological preparations demonstrated schizonts associated with macrophages present within blood vessels or associated with the vascular wall. Organisms were most frequently noted in lung, liver and spleen samples.

**Table 3** Days post infestation when *Cytauxzoon felis* was first detected in non-treated control cats. Cats were infested with *Amblyomma americanum* adults acquisition fed as nymphs on the *Cytauxzoon felis* donor cat

| Days post infestation with *C. felis* acquisition fed *A. americanum* | NRB5 | ZAK3 | OIF5 | ZAI4 | HIG6 | GRI4 | ZAM3 | GRG2 | NRH1 | GRF6 |
|---|---|---|---|---|---|---|---|---|---|---|
| –38 | – | – | – | – | – | – | – | – | – | – |
| 0 | – | – | – | – | – | – | – | – | – | – |
| 8 | – | – | – | – | – | – | – | + | – | – |
| 10 | – | – | – | – | – | – | – | | – | – |
| 12 | + | – | – | – | – | – | – | | – | + |
| 14 | | – | – | – | – | + | + | | + | |
| 16 | | + | – | + | + | | | | | |
| 60 | | | – | | | | | | | |

## Discussion

The availability of acaricides approved for use on cats is limited compared to those of dogs (Blagburn and Dryden 2009). Our results indicate that the 10% imidacloprid/4.5% flumethrin collar prevented *C. felis*-infected *A. americanum* adults from attaching and feeding on cats. Because infected ticks did not attach, transmission of *C. felis* to cats was prevented. Effectiveness of this collar for preventing and treating flea and tick infestations on cats has been demonstrated (Fourie et al. 2012; Stanneck et al. 2012b; Stanneck et al. 2012c). Application of this collar on cats has also been effective for preventing the transmission of *Bartonella henselae* by *Ctenocephalides felis* (Lappin et al. 2013). To the authors' knowledge, our study is the first to report that application of an approved acaricide can prevent the transmission of a tick-borne disease agent to cats.

Imidacloprid is a chloronicotinyl compound that has insecticidal properties coupled with low mammalian toxicity and is aimed at treating and controlling fleas (Stanneck et al. 2012b). Additionally, imidacloprid has also been used in combination with moxidectin for the treatment, prevention, and control of *Aelurostrongylus abstrusus* (Traversa et al. 2009), *Capillaria aerophila* (Traversa et al. 2012), *Dirofilaria immitis* (Arther et al. 2003; Arther et al. 2005; Arther et al. 2007), *Otodectes cynotis* (Fourie et al. 2003; Davis et al. 2007; Farkas et al. 2007) and *Toxocara cati* (Reinemeyer and Charles 2003) in cats. Flumethrin is an α-cyano-pyrethroid and is effective as an acaricide (Stanneck et al. 2012b). Activity of flumethrin is mediated through voltage-gated sodium channels in neural tissue causing them to remain open longer than physiologically normal, therefore extending the period of sodium influx resulting in death of acarines (Stanneck et al. 2012a). Cats are typically considered to have a reduced enzyme pattern for hydrolysis of pyrethroid-esters resulting in toxic metabolites developing during the pyrethroid degradation process. However, the metabolism of flumethrin in cats does not require glucoronidation, allowing it to be safely used as an acaricide (Stanneck et al. 2012c).

Peer-reviewed surveys reporting the occurrence or prevalence of ticks on cats in the United States are lacking. Akucewich et al. (2002) reported that 5 of 200 (2.5%) feral cats sampled during the summer from North-central Florida were infested with one adult female *Rhipicephalus sanguineus*, one female and one male *A. americanum*, five female *D. variabilis* and one female *Ixodes scapularis*. In the authors' experience, *A. americanum* followed by *D. variabilis* and *I. scapularis* are the most common ticks recovered off cats from early

spring through fall in the South, South-central, and southeastern United States where cytauxzoonosis is enzootic. Even though cats are fastidious groomers and can remove ticks through daily cleaning activities, our study demonstrated that all cats were susceptible to infestation with *A. americanum*. Furthermore, non-treated control cats were persistently infested with ticks during the efficacy trial, despite unrestricted grooming, and replete *A. americanum* females were recovered from each of the non-treated control cats (data not shown).

*Cytauxzoon felis* is an emerging infectious disease agent in cats and cases of cytauxzoonosis in domestic cats are likely to be found in geographical areas outside of the currently recognised distribution. Surveys to determine the prevalence of *C. felis* infection in wild felids have demonstrated that the parasite occurs in bobcats (*Lynx rufus*) in geographical areas outside of the currently recognised distribution in domestic cats (Birkenheuer et al. 2008; Shock et al. 2011). Ecological niche modelling of bobcats and *A. americanum* suggests a broader distribution of *C. felis* than what was predicted from models based solely on locations of *C. felis* infection in domestic cats (Mueller et al. 2013).

Previous *A. americanum* transmission studies detected *C. felis* in one cat at 11 d.p.i. (Reichard et al. 2009) and in 4 cats at 18 and 19 d.p.i. (Reichard et al. 2010). In the current study, *C. felis* was transmitted to 9 of 10 (90%) non-treated control cats with *C. felis* first detected by PCR amplification between 8 and 16 d.p.i. Clinical signs of cytauxzoonosis were not observed in any of the 9 *C. felis*-infected cats, nor did any of these cats have a detectable parasitaemia based on light microscopy of stained blood films, when infections were first detected by PCR amplification. Detecting transmission of *C. felis* 8 d.p.i. is the shortest prepatent period reported to date and suggests that PCR can be of diagnostic value to detect acute infections prior to the onset of clinical disease.

The minimum burden of *C. felis*-infected *A. americanum* to establish infection in cats is not known. Nor is it known how long a *C. felis*-infected tick must feed on a cat for transmission to occur. In the current study, *C. felis* was transmitted to cats with tick burdens ranging from as few as 7 (on cat GRF6) to as many as 28 (on cat ZAI4) at 48 hours post infestation. Conversely, cat OIF5 did not become infected with *C. felis* after attachment of 15 infected *A. americanum* at 48 hours post infestation. PCR analysis on a subset of the *C. felis*-infected *A. americanum* used for the efficacy trial indicated that the prevalence of infection was 4%. The prevalence of *C. felis* in wild-caught, flat *A. americanum* from a region enzootic for cytauxzoonosis was 0.8% in nymphs, 0.5% in adult males and 1.5% in adult females (Reichard et al. 2010).

Application of the 10% imidacloprid/4.5% flumethrin collar prevented *A. americanum* from attaching and feeding on treated cats preventing transmission of *C. felis*. However, application of an effective acaricide is just one component of an integrated management plan that should be employed for treatment, control and prevention of ticks and tick-borne disease agents in cats.

## Ethical standards

All animal experiments were approved by responsible local administrations and were conducted in accordance with local regulations regarding animal welfare.

## Conflict of interest

The present study was funded in by Bayer Animal Health GmbH. Logistic and laboratory support was provided by the Animal Resources Unit and the Center for Veterinary Health Sciences at Oklahoma State University. Robert G Arther, Joseph A Hostetler, and Kara L Raetzel are employed by Bayer Health Care, Animal Health.

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