



# Minimum transmission time of *Cytauxzoon felis* by *Amblyomma americanum* to domestic cats in relation to duration of infestation, and investigation of ingestion of infected ticks as a potential route of transmission

Jennifer E Thomas<sup>1\*</sup>, Cameon M Ohmes<sup>2</sup>, Mark E Payton<sup>3</sup>, Joseph A Hostetler<sup>2</sup> and Mason V Reichard<sup>1</sup>

Journal of Feline Medicine and Surgery  
2018, Vol. 20(2) 67–72  
© The Author(s) 2017



Reprints and permissions:  
sagepub.co.uk/journalsPermissions.nav  
DOI: 10.1177/1098612X17691172  
journals.sagepub.com/home/jfms

This paper was handled and processed by the American Editorial Office (AAFP) for publication in *JFMS*



## Abstract

**Objectives** The objectives of the present study were to determine the duration of infestation by *Amblyomma americanum* necessary for transmission of *Cytauxzoon felis* to domestic cats and to determine if ingestion of *C felis*-infected *A americanum* by cats is a route of transmission.

**Methods** Forty-nine cats were assigned to one of seven groups, with seven cats per group. Cats were infested with *A americanum* adults, acquisition fed as nymphs on a cytauxzoonosis survivor cat, for 12 h (group 1), 18 h (group 2), 24 h (group 3), 36 h (group 4), 48 h (group 5) and to repletion (group 7; control). Cats in group 6 were fed *C felis*-infected ticks. Thumb counts were performed at the end of the duration of infestation for groups 1–5 and at 48 h for the control group. For group 6, 50 live *C felis*-infected adult *A americanum* were mixed with food and fed to each of the cats. Transmission of *C felis* was determined by examining blood of cats by DNA extraction followed by PCR.

**Results** Of 50 ticks placed on each cat (groups 1–5 and 7), the arithmetic mean attachment  $\pm$  SEM ranged from  $46.9 \pm 1.9$  in group 3 to  $49.3 \pm 0.3$  in group 1. In group 6, the average number  $\pm$  SEM of ticks ingested was  $46.5 \pm 2.3$ . One cat in group 5 that had been infested for 48 h became infected with *C felis*. None of the cats in group 6 (ingestion) became infected with *C felis*. Six of 7 (85.7%) cats in group 7, the control group, became infected with *C felis*.

**Conclusions and relevance** Our results indicate that transmission of *C felis* to domestic cats can happen as quickly as  $>36$  h but  $\leq 48$  h of exposure to *A americanum* infected with *C felis* and that ingestion of *C felis*-infected *A americanum* is not a likely route of transmission.

**Accepted:** 5 January 2017

## Introduction

*Cytauxzoon felis* is an emerging tick-transmitted apicomplexan that causes severe disease in domestic cats. Cats with cytauxzoonosis are often febrile, dehydrated, emaciated, anorexic and icteric.<sup>1,2</sup> Clinical signs begin approximately 11 days after being bitten by an infected tick.<sup>2</sup> As clinical signs appear after ticks have had enough time to feed to repletion, vectors may no longer be present on ill cats when they present to veterinary clinics. The absence of ticks on ill cats should not limit or reduce a practitioner's index of suspicion of cytauxzoonosis.

<sup>1</sup>Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA

<sup>2</sup>Bayer Animal Health, LLC, Shawnee Mission, KS, USA

<sup>3</sup>Department of Statistics, Oklahoma State University, Stillwater, OK, USA

\*Current address: Department of Clinical Sciences, Texas A & M University, TX, USA

### Corresponding author:

Mason V Reichard MS, PhD, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, 250 McElroy Hall, Stillwater, OK 74078, USA  
Email: mason.reichard@okstate.edu

Both *Dermacentor variabilis* (American dog tick)<sup>3,4</sup> and *Amblyomma americanum* (lone star tick)<sup>2,5</sup> have been shown to be competent vectors of *C felis* to domestic cats. However, *A americanum* has shown to be a more reliable vector under laboratory conditions.<sup>2,5</sup> In the south-central USA most cases of cytauxzoonosis are observed in late spring or early summer, followed by a second but smaller uptick in cases during the fall months.<sup>6</sup> The bimodal pattern of cytauxzoonosis cases throughout the year likely reflects a slight delay in the questing activity and host-seeking behaviors of tick vectors. Cats living in low-density residential areas, urban edge habitat, wooded cover and in closer proximity to natural or unmanaged areas (ie, areas likely to harbor ticks and vertebrate reservoir hosts) were at higher risk of *C felis* infection.<sup>6</sup>

Transmission of *C felis* is dependent upon infected ticks taking a blood meal from a feline host. Control strategies have focused on limiting cats' access to tick-infested areas,<sup>7,8</sup> or application of an acaricide to prevent ticks from feeding on cats.<sup>9</sup> Currently, four acaricides are approved for use on cats in the USA: etofenprox, fipronil, fluralaner and flumethrin. Delineating the transmission time of *C felis* from *A americanum* to cats is important as acaricides have different mechanisms of action,<sup>10</sup> speed of kill and repellency. Indeed, anecdotal reports suggest that *C felis* may be transmitted to ticks, despite routine application of fipronil,<sup>7,8</sup> whereas application of an imidacloprid/flumethrin collar prevented the transmission of *C felis* to cats by *A americanum*.<sup>9</sup>

A previous study demonstrated the duration of infestation (DOI) necessary for transmission of *C felis* by *A americanum* was 72 h.<sup>11</sup> However, we hypothesized that transmission of *C felis* occurred in less than 72 h, considering the reports of cats contracting cytauxzoonosis, despite prophylactic treatment with fipronil, a compound with a labeled 48 h speed of kill for ticks.<sup>12</sup> Additionally, as most cats are fastidious groomers and are thought to ingest ticks, we speculated that cats could become infected with *C felis* through ingesting infected *A americanum*. The objectives of the present study were to determine if transmission of *C felis* occurred before 72 h of infestation with *A americanum* and to ascertain if ingestion of *C felis*-infected *A americanum* by cats is a route of transmission.

## Materials and methods

### Study design

This study was an unmasked, randomized, controlled block study with an individual cat as the experimental unit. There were seven groups each, comprised of seven principal cats (Table 1). Cats were either infested or fed 25 pairs (50 total) of *C felis*-acquisition-fed *A americanum*. To determine the duration of infestation necessary for *C felis* transmission to cats, ticks on infested cats were

**Table 1** Grouping and treatment of principal cats

Group	Tick exposure	Number of cats
Group 1	12 h infestation	7
Group 2	18 h infestation	7
Group 3	24 h infestation	7
Group 4	36 h infestation	7
Group 5	48 h infestation	7
Group 6	Ingestion of ticks	7
Group 7	Fed to repletion	7

allowed to feed for 12 h  $\pm$  30 mins (group 1), 18 h  $\pm$  30 mins (group 2), 24 h  $\pm$  30 mins (group 3), 36 h  $\pm$  30 mins (group 4) or 48 h  $\pm$  30 mins (group 5), after which they were removed from infested cats. To determine if cats could become infected through ingestion of infected ticks, ticks were fed with canned food to cats (group 6). In the control group, ticks were allowed to feed until repletion (group 7) and detached/fell off on their own. Infection of *C felis* was determined by monitoring the cats for clinical signs suggestive of cytauxzoonosis and by PCR analysis of blood samples collected throughout the study.

### Donor and principal cats

All procedures were reviewed and approved by the Institution Animal Care and Use Committee at Oklahoma State University. Two *C felis* donor cats maintained in the Animal Resources unit at Oklahoma State University (Stillwater, OK, USA) were used to acquisition feed *A americanum* nymphs. The 49 principal cats used for transmission feeding of ticks and fed *C felis*-infected ticks were purpose-bred Class A research cats purchased through a commercial supplier (Liberty Research). All cats used were domestic shorthairs. All principal cats tested negative for *C felis* infection by PCR (described below) prior to study initiation.

### Cat infestations, cat ingestion of ticks and tick counts

*A americanum* nymphs were purchased from the Tick Rearing Facility at Oklahoma State University. These nymphs were acquisition fed on two *C felis* donor cats. The donor cats were anesthetized via intramuscular injection with tiletamine HCl and zolazepam HCl (Telazol; Zoetis) at 4.0–12 mg/kg and their torsos shorn. Nymphs were placed between the shoulder blades of the cats. Knit cotton tubes that encompassed the cats' thorax and abdomen were used to help confine and minimize escape of the ticks during feeding, as well as prevent donor cats from grooming ticks during acquisition feeding. Engorged nymphs were collected daily, placed into paper cartons and stored in a humidity chamber maintained with 90–98% relative humidity at 20–25°C with a 14 h light/10 h dark photoperiod while they molt to

adults. Adult *A americanum* acquisition fed with *C felis* were enumerated and transmission fed on the 49 principal cats.

Transmission trials were performed using principal cats infested with 25 pairs (50 total) of adult *A americanum* that were acquisition fed as nymphs on the *C felis* donor cat. Infestations on principal cats were performed in a similar manner as described for the *C felis* donor. Permitted DOIs by *A americanum* on the principal study cats were 12 h  $\pm$  30 mins (group 1), 18 h  $\pm$  30 mins (group 2), 24 h  $\pm$  30 mins (group 3), 36 h  $\pm$  30 mins (group 4), 48 h  $\pm$  30 mins (group 5) and to repletion (group 7, control). At each of these respective time points, cats in groups 1–5 were anesthetized with Telazol and all ticks, attached and non-attached, were removed from cats, enumerated and placed in 70% ethanol. Group 7 cats were sedated with Telazol at 48 h  $\pm$  60 mins and ticks were enumerated but not removed ('thumb counts'), after which these group 7 cats were returned to standard housing and ticks were allowed to feed until repletion.

Cats in group 6 were fed 50 adult *A americanum* that had been acquisition fed on the *C felis* donor cats. Briefly, 25 male and 25 female ticks were mixed with canned cat food and fed to principal cats in group 6. Cats were monitored for 1 h after feeding, to determine if cats vomited any of the canned food or ticks. After 1 h the remaining cat food was examined and ticks that were not ingested were quantified. Ticks fed to cats in group 6 came from the same batch of acquisition-fed *A americanum* adults used for transmission studies in groups 1–5 and 7, and overlapped in time with *C felis* transmission trials. As feeding of infected *A americanum* is considered the primary route of *C felis* transmission to domestic cats, cats in group 7 were used as controls for the group 6 cats that were fed *C felis*-infected ticks, as well as groups 1–5.

#### Determination of *C felis* infection

Cats were observed daily for clinical signs of cytauxzoonosis until 30 days postinfestation. Blood samples were collected periodically throughout the study. Up to 1 ml of blood was collected from the jugular vein by sterile collection. Blood was stored at 4°C until processing for analysis of *C felis* infection. In addition to observing for clinical signs consistent with cytauxzoonosis, *C felis* infection was determined by DNA extraction and PCR amplification. DNA was extracted from 200  $\mu$ l whole blood, using a commercially available genomic DNA extraction kit (GeneJET Whole Blood Genomic DNA Purification; Thermo Scientific) according to the manufacturer's instructions. Infection with *C felis* in the donor cat was confirmed through primary PCR amplification performed on an aliquot of purified DNA, using previously described methods.<sup>13</sup> A nested PCR amplification was also performed using previously described methods.<sup>2</sup>

#### Statistical methods

Descriptive statistics, including the geometric mean, arithmetic mean, SEM and range, were used to quantify tick attachment and cat ingestion of ticks. The DOI necessary for acquisition-fed *A americanum* transmission of *C felis* to domestic cats was modeled using binary response regression. The analysis of the proportion of cats found positive from multiple blood draws among study groups was analyzed using Fisher's exact tests. An alpha value of 0.05 was assumed and SAS 9.2 statistical software was used. Data for tick attachment (secondary variable) were transformed using an angular or arcsine square root transformation to alleviate issues associated with percentage data and heterogeneity of variance.

## Results

#### Tick attachment and ingestion

The number of ticks attached to cats in the treatment and control groups was excellent (Table 2). The lowest number of ticks attached was in group 3, with a geometric mean of 46.6. The highest number of ticks attached was in group 1, with a geometric mean of 49.3. A significant difference in the number of ticks attached to cats among groups was not detected (F-value = 0.58, df = 5,  $P = 0.7113$ ). The number of ticks ingested by cats in group 6 was also high. The geometric mean, arithmetic mean  $\pm$  SEM and range of ticks ingested by cats in group 6 were 45.0, 45.5  $\pm$  2.3 and 36–50, respectively.

#### Infection of *C felis*

None of the cats in groups 1, 2, 3, 4 or 6 became infected with *C felis* (Table 3). One cat (14%) in group 5 (48 h) became infected with *C felis* at 12 days after infestation. Six cats (86%) in group 7 (control group) became infected with *C felis* (Table 3) at 8, 8, 10, 12, 12 and 12 days after infestation, respectively. Significantly more cats in the control group ( $P = 0.0047$ ) became infected with *C felis* than cats in groups 1, 2, 3, 4 and 6. Similarly, more cats ( $P = 0.0291$ ) in the control group became infected with *C felis* than in group 5. All cats infected with *C felis* were determined by PCR before the onset of clinical signs cytauxzoonosis. Cats infected with *C felis* were transferred to another protocol where they were eligible to be treated for cytauxzoonosis.

## Discussion

The life cycle of *C felis* in *A americanum* is not known. Generally speaking, a short period of time is usually necessary from when ticks attach to hosts and begin to feed to when pathogens are transmitted. During this delay it is thought that pathogens move from a state of dormancy or are initiated to continue their development and progress through stages of their life cycle.<sup>14–20</sup> Transfer of pathogens from ticks to hosts is thought to occur across a continuum

**Table 2** Mean attachment of *Amblyomma americanum* adults, acquisition fed as nymphs on *Cytauxzoon felis* survivor donor cats, on principal cats in groups 1–5 and 7

Tick attachment	Statistic	Experimental group					
		1	2	3	4	5	7
Not attached	Geometric mean	NA	NA	NA	NA	NA	1.1
	Arithmetic mean $\pm$ SEM	0.6 $\pm$ 0.2	1.6 $\pm$ 0.9	1.6 $\pm$ 0.8	2.6 $\pm$ 1.3	1.6 $\pm$ 0.8	1.1 $\pm$ 0.1
	Minimum–maximum	0–1	0–7	0–6	0–9	0–6	1–2
Attached	Geometric mean	49.3	48.0	46.6	46.7	47.7	48.1
	Arithmetic mean $\pm$ SEM	49.3 $\pm$ 0.3	48.0 $\pm$ 1.1	46.9 $\pm$ 1.9	46.9 $\pm$ 1.3	47.9 $\pm$ 1.1	48.1 $\pm$ 0.3
	Minimum–maximum	48–50	42–50	36–50	41–50	42–50	47–49

NA = not applicable

**Table 3** Number of cats infected with *Cytauxzoon felis*

Group	Number of cats infected with <i>C felis</i>	Number of cats not infected with <i>C felis</i>	Total number of cats
1 (12 h infestation)	0	7	7
2 (18 h infestation)	0	7	7
3 (24 h infestation)	0	7	7
4 (36 h infestation)	0	7	7
5 (48 h infestation)	1	6	7
6 (ingestion of ticks)	0	7	7
7 (control group)	6	1	7
Total	7	42	49

whereby a few pathogens are transmitted early with the probability of transmission increasing over the DOI.<sup>14,18</sup> A notable exception to the reactivation paradigm is Powassan virus. Ebel and Kramer demonstrated transmission of Powassan virus from *Ixodes scapularis* to mice after 15 mins of attachment.<sup>15</sup>

*Theileria parva*, a piroplasm closely related to *C felis*,<sup>21</sup> is transmitted to cattle by *Rhipicephalus appendiculatus*. Ochanda et al demonstrated that the time necessary for transmission of *T parva* was affected, in part, by the ambient temperatures in which the ticks were held prior to infestation on cattle.<sup>19</sup> *R appendiculatus* infected with *T parva* held at 18°C (64.4°F) for 6 days and 85% relative humidity prior to infestation transmitted the pathogen in  $\leq 72$  h. Ticks held at 37°C (98.6°F) for 6 days and 85% relative humidity prior to infestation transmitted *T parva* in  $\leq 24$  h. In the current study, *A americanum* acquisition fed as nymphs on a cytauxzoonosis survivor cat were incubated at 20–25°C (60–77°F) and 90–98% relative humidity prior to transmission feeding on principal cats. None of the principal cats infested for 12, 18, 24 or 36 h became infected, whereas 1/7 (14%) cats became infected with *C felis* after 48 h of infestation, and 6/7 (86%) control cats in which *A americanum* were allowed to feed until repletion became infected. Our results indicated that the minimum amount of time for transmission of *C felis* with *A americanum* adults maintained at room temperature is

$>36$  h to  $\leq 48$  h. In Oklahoma where *C felis* is enzootic and the current study was performed, the average high temperature between April and October ranges from 22.4°C to 34.4°C (72–94°F) and average low temperature from 9.8°C to 22.3°C (50–72°F).<sup>22</sup> Moreover, in July and August the average low and high temperatures range from 21.6–34.4°C (71–94°F). That is, for 2 months of the year, ambient temperatures in an area enzootic for *C felis* are at or above the temperature at which the current study was conducted. It has yet to be determined if increasing the ambient temperature shortens the transmission time of *C felis* other than what was demonstrated in the current study.

*Babesia microti*, another piroplasm related to *C felis*,<sup>21</sup> is transmitted to rodents and humans by the bite of infected *I scapularis*. Piesman and Spielman used *I scapularis* nymphs acquisition fed as larvae on a hamster infected with *B microti*, to determine the transmission time of *B microti*.<sup>17</sup> They demonstrated that 9% (1/11) of the hamsters became infected with *B microti* after 36 h of attachment, 17% (2/12) were infected after 48 h and 50% (6/12) were infected with *B microti* after 54 h of attachment. In the current study, 14% (1/7) of cats became infected with *C felis* after 48 h of infestation, whereas none of the cats in the 12, 18, 24 or 36 h groups became infected.

Factors that govern the transmission of pathogens from ticks to vertebrate host are not well understood. We can only speculate as to the effects that doses of

pathogens in ticks, vertebrate host immune responses to ticks and to pathogens, strain variation of pathogen and extrinsic environmental variables, among a few, have on the transmission time of pathogens to vertebrate hosts through ticks. Future research should explore the effects of increasing ambient temperature on *C felis* transmission from *A americanum* to cats, to determine if the minimum transmission time can be quicker than what was demonstrated in the current study. Similarly, intrastadial transmission of infected ticks from an infested cat or other vertebrate host to a naive cat should also be explored as a mechanism for which the minimum transmission time may be shorter than what was demonstrated, as pathogen replication would already be initiated and a reactivation period would not be necessary.

Acaricides approved for use on cats in the USA include etofenprox, fipronil, flumethrin and fluralaner. Etofenprox and flumethrin are pyrethroids that kill and repel ticks.<sup>23</sup> Fipronil is a phenylpyrazole and does not have repellent properties against ticks. Fluralaner is an isoxazoline and does not have repellent properties against ticks. Fourie et al compared the acaricidal efficacy of two combination products used to control ticks on cats.<sup>24</sup> They demonstrated that flumethrin 4.5% (w/w)/imidacloprid 10% (w/w) was 100% effective against *Ixodes ricinus* adults on cats at 6, 12, 24 and 48 h postinfestation, whereas fipronil 8.3% (w/v)/(S)-methoprene 10% (w/v)/eprinomectin 0.4% (w/v)/praziquantel 8.3% (w/v) was 0–16%, 26.8–50%, 31.5–81.5% and 83.2–100% at 6, 12, 24 and 48 h postinfestation, respectively. Reichard et al demonstrated that application of flumethrin 4.5% (w/w)/imidacloprid 10% (w/w) prevented the transmission of *C felis* by adult *A americanum*,<sup>9</sup> which demonstrated, for the first time, that transmission of a tick-borne pathogen of cats could be blocked using a repellent.

Vertebrate hosts becoming infected with tick-borne protozoa by ingestion of infected ticks is not novel. Dogs can become infected with *Hepatozoon americanum* through ingestion of infected *Amblyomma maculatum*,<sup>25,26</sup> and *Hepatozoon canis* by ingestion of infected *Rhipicephalus sanguineus*.<sup>27</sup> As most cats are considered to be excellent groomers, we sought to determine if cats could become infected with *C felis* through ingestion of infected *A americanum*. We fed up to 50 *A americanum* acquisition fed as nymphs on a *C felis* donor to seven principal cats (group 6). None of the cats in this tick ingestion group became infected with *C felis*, whereas 6/7 cats in the control group did become infected with *C felis* suggesting that ingestion of *C felis*-infected *A americanum* adults is not a likely route of transmission to domestic cats.

## Conclusions

Cytauxzoonosis is a severe and often fatal disease of domestic cats. Cats become infected via the bite of an

infected *A americanum* or possibly *Dermacentor variabilis*. Results from the current study suggest that ingestion of *C felis*-infected *A americanum* is not a likely route of transmission to naive cats. Conversely, transmission of *C felis* can happen as quickly as 48 h, with *A americanum* adults held at room temperature for at least 6 days before infesting cats. It is not known what effect increasing the ambient temperature of ticks or intrastadial transmission of *C felis* has on the transmission time to domestic cats. The rapid transmission of *C felis* to cats in >36 to ≤48 h emphasizes the need of having cats on approved and effective acaricides to help prevent ticks from attaching and feeding.

**Conflict of interest** CMO and JAH were full-time employees of Bayer HealthCare Animal Health at the time of the study. In the past 5 years, JET and MVR have received honoraria and research support from multiple veterinary pharmaceutical companies for related and unrelated activities.

**Funding** This research was funded through a grant from Bayer HealthCare Animal Health.

## References

- Wagner JE. A fatal cytauxzoonosis-like disease in cats. *J Am Vet Med Assoc* 1976; 168: 585–588.
- Reichard MV, Edwards AC, Meinkoth JH, et al. Confirmation of *Amblyomma americanum* (Acari: Ixodidae) as a vector for *Cytauxzoon felis* (Piroplasmorida: Theileriidae) to domestic cats. *J Med Entomol* 2010; 47: 890–896.
- Blouin EF, Kocan AA, Glenn BL, et al. Transmission of *Cytauxzoon felis* Kier, 1979 from bobcats, *Felis rufus* (Schreber), to domestic cats by *Dermacentor variabilis* (Say). *J Wildl Dis* 1984; 20: 241–242.
- Blouin EF, Kocan AA, Kocan KM, et al. Evidence of a limited schizogonous cycle for *Cytauxzoon felis* in bobcats following exposure to infected ticks. *J Wildl Dis* 1987; 23: 499–501.
- Reichard MV, Meinkoth JH, Edwards AC, et al. Transmission of *Cytauxzoon felis* to a domestic cat by *Amblyomma americanum*. *Vet Parasitol* 2009; 161: 110–115.
- Reichard MV, Baum KA, Cadenhead SC, et al. Temporal occurrence and environmental risk factors associated with cytauxzoonosis in domestic cats. *Vet Parasitol* 2008; 152: 314–320.
- Meinkoth JH and Kocan AA. Feline cytauxzoonosis. *Vet Clin North Am Small Anim Pract* 2005; 35: 89–101.
- Sherrill MK and Cohn LA. Cytauxzoonosis: diagnosis and treatment of an emerging disease. *J Feline Med Surg* 2015; 17: 940–948.
- Reichard MV, Thomas JE, Arther RG, et al. Efficacy of an imidacloprid 10%/flumethrin 4.5% collar (Seresto, Bayer) for preventing the transmission of *Cytauxzoon felis* to domestic cats by *Amblyomma americanum*. *Parasitol Res* 2013; 112: S11–S20.
- Plumb DC. Plumb's veterinary drug handbook. 7th ed. Ames, IA: Wiley-Blackwell, 2011.

- 11 Nagamori Y. **Transmission of *Cytauxzoon felis* by *Amblyomma americanum*: engorgement weight of nymphs and attachment time of adults for transmission to domestic cats.** MS Thesis, Oklahoma State University, 2016.
- 12 Cruthers L, Slone RL, Guerrero AJ, et al. **Evaluation of the speed of kill of fleas and ticks with Frontline Top Spot in dogs.** *Vet Ther* 2001; 2: 170–174.
- 13 Bondy PJ, Cohn LA, Tyler JW, et al. **Polymerase chain reaction detection of *Cytauxzoon felis* from field-collected ticks and sequence analysis of the small subunit and internal transcribed spacer 1 region of the ribosomal RNA gene.** *J Parasitol* 2005; 91: 458–461.
- 14 des Vignes F, Piesman J, Heffernan R, et al. **Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs.** *J Infect Dis* 2001; 183: 773–778.
- 15 Ebel GD and Kramer LD. **Short report: duration of tick attachment required for transmission of powassan virus by deer ticks.** *Am J Trop Med Hyg* 2004; 71: 268–271.
- 16 Katavolos P, Armstrong PM, Dawson JE, et al. **Duration of tick attachment required for transmission of granulocytic ehrlichiosis.** *J Infect Dis* 1998; 177: 1422–1425.
- 17 Piesman J and Spielman A. **Human babesiosis on Nantucket Island: prevalence of *Babesia microti* in ticks.** *Am J Trop Med Hyg* 1980; 29: 742–746.
- 18 Piesman J, Mather TN, Sinsky RJ, et al. **Duration of tick attachment and *Borrelia burgdorferi* transmission.** *J Clin Microbiol* 1987; 25: 557–558.
- 19 Ochanda H, Young AS, Mutugi JJ, et al. **The effect of temperature on the rate of transmission of *Theileria parva parva* infection to cattle by its tick vector, *Rhipicephalus appendiculatus*.** *Parasitol* 1988; 97: 239–245.
- 20 Spencer RR and Parker RR. **Rocky mountain spotted fever: infectivity of fasting and recently fed ticks.** *Public Health Rep* 1923; 38: 333–339.
- 21 Lack JB, Reichard MV and Van Den Bussche RA. **Phylogeny and evolution of the Piroplasmida as inferred from 18S rRNA sequences.** *Int J Parasitol* 2012; 42: 353–363.
- 22 Data UC. **Climate Oklahoma City – Oklahoma and Weather averages Oklahoma City – Weather history January 2016.** <http://www.usclimatedata.com/climate/oklahoma-city/oklahoma/united-states/usok0400> (accessed October, 25 2016).
- 23 Bowman DD. **Georgis' parasitology for veterinarians.** 10th ed. St Louis, MO: Saunders/Elsevier, 2013.
- 24 Fourie JJ, Horak IG, de Vos C, et al. **Comparative speed of kill, repellent (anti-feeding) and acaricidal efficacy of an imidacloprid/flumethrin collar (Seresto) and a fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on (Broadline) against *Ixodes ricinus* (Linne, 1758) on cats.** *Parasitol Res* 2015; 114: S109–S116.
- 25 Mathew JS, Ewing SA, Panciera RJ, et al. **Experimental transmission of *Hepatozoon americanum* Vincent-Johnson et al., 1997 to dogs by the Gulf Coast tick, *Amblyomma maculatum* Koch.** *Vet Parasitol* 1998; 80: 1–14.
- 26 Garrett JJ, Kocan AA, Reichard MV, et al. **Experimental infection of adult and juvenile coyotes with domestic dog and wild coyote isolates of *Hepatozoon americanum* (Apicomplexa: Adeleorina).** *J Wildl Dis* 2005; 41: 588–592.
- 27 Baneth G, Samish M and Shkap V. **Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*).** *J Parasitol* 2007; 93: 283–299.