Parasitol Res (2013) 112:S21–S32 DOI 10.1007/s00436-013-3278-6

Imidacloprid 10%/Flumethrin 4.5% Collars (Seresto®, Bayer) Successfully Prevent Long-Term Transmission of *Ehrlichia canis* by Infected *Rhipicephalus sanguineus* Ticks to Dogs

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Abstract

The objective of this study was to determine the empirical efficacy of imidacloprid 10%/flumethrin 4.5% (Seresto®) collars in preventing long-term transmission of *Ehrlichia canis* by infected *Rhipicephalus sanguineus* ticks to dogs. The study was a parallel group design, single centre, randomised, non-blinded, controlled, long-term efficacy study. The treatment group of 8 dogs was fitted with Seresto® collars, the untreated control group of 8 dogs received no collars. *Ehrlichia canis*-infected ticks were released into the dogs sleeping quarters at 14-day intervals up to Day +378. Control group dogs infected with *E. canis* were continuously replaced to keep the control sample size constant, and a total of 39 control dogs were required. The final clinical examination and blood sampling occurred on Day +420. The primary assessment criterion was the number of dogs infected with *E. canis,* as confirmed by IFA and PCR, and the secondary criterion was the acaricidal efficacy based on tick counts. All scheduled blood samples taken were subject to analyses for both PCR and IFA, but only positive cases are discussed. Up to Day +378, none of the collar-treated dogs were infected with *E. canis*, whereas 34 of the 35 untreated dogs enrolled before Day +371 were infected. The acaricidal efficacy of the collar ranged from 90% to 100% for the duration of the assessment period.

Introduction

Ticks are common ectoparasites present in many areas of the world. *Rhipicephalus sanguineus* is the most common tick species in subtropical and tropical regions as well as being one of the main European tick species (Otranto et al. 2008; Stanneck et al. 2012). *R. sanguineus* is responsible for the transmission of *Ehrlichia canis* to dogs, which causes canine monocytic ehrlichiosis (CME), the most well-known globally occurring canine tick-borne disease (Otranto et al. 2008). Dogs with CME present a wide range of clinical symptoms, including weight loss, lethargy, anorexia, pyrexia, lymphadenomegaly and splenomegaly (Harrus et al. 1997). Occular signs such as corneal opacity may also occur and subretinal haemorrhages can lead to blindness (Harrus et al. 1998).

The imidacloprid 10%/flumethrin 4.5% collar (Seresto®) contains a combination of the acaricide flumethrin with the insecticide imidacloprid. Flumethrin is a highly potent acaricide, which has been registered for animal use since 1986 and has been used as an active ingredient in the companion animal product "Kiltix®" collar (propoxur 10%/flumethrin 2.25%) for over 15 years (Liebisch et al. 1996). The newer Seresto® collar has been available commercially since 2012 and the active ingredients have been shown to spread from the collar over the entire skin surface of the treated animal (Stanneck et al. 2012a).

The imidacloprid 10%/flumethrin 4.5% collar has been shown to be safe and highly effective in the treatment and prevention of tick and flea infestations in cats and dogs. It has been proven to reduce tick counts by at least 90% in cats and dogs under field conditions for a period of at least 7–8 months (Stanneck et al. 2012b). Though the time frame of *E. canis* transmission by *R. sanguineus* ticks was not part of this study, it is known from current literature that rickettsial pathogens can be transmitted from ticks to dogs within a short time frame of just 4 hours (Nicholson et al. 2010). It is therefore important to determine whether the imidacloprid 10%/flumethrin 4.5% collars act fast enough to prevent *R. sanguineus* ticks from infecting dogs with *E. canis*. The aim of this study was to empirically evaluate the long-term efficacy of the Seresto® collar formulation in preventing the transmission of *E. canis* to dogs by infected *R. sanguineus* ticks.

Materials and methods

General study design

This study was conducted in South Africa and lasted from December 2011 to February 2013; the last blood collection and clinical examination was on Day +420. The study was a parallel group design, single centre, randomised, non-blinded, controlled, long-term efficacy study conducted on two groups each containing eight dogs. Untreated control group dogs diagnosed with CME during the study were replaced to ensure that the control group population remained constant for the duration of the experimental phase. The sample size of eight dogs per group is compliant with the CVMP guideline "Guidelines for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestations in Dogs and Cats".

Acclimatisation, ranking, collar administration and clinical examinations were performed at the time intervals shown in Table 1. Collars were replaced at Day 155 to provide a full year of collar protection during the study with the second treatment period ending after the collars' registered 8 months maximum efficacy period. In order not to have the last month of collar efficacy in the middle of the study, an earlier retreatment after 155 days instead of 8 months was chosen. The approximate one-year study duration was designed to mimic an animal under full-year protection in a hyperendemic area with tick exposure throughout the whole year, as it may occur in southern Mediterranean areas.

Table 1 Study overview

Acclimatisation	Ranking and allocations to groups	Collar administration to group 2	Clinical examinations
Days –7 to –1	Day –2	Day 0 and +155	Days –7 +28, +42, +56, +70, +84, +98, +112, +126, +140, +154, +168, +182, +196, +210, +224, +238, +252,+266, +280, +294, +308, +322, +336, +350,+364, +378, +392, +406 and +420

Table 2 Time schedule of tick challenges and *in situ* tick counts

Artificial tick challenges	Assessments *in situ* tick counts
Days +14, +28, +42, +56, +70, +84, +98, +112, +126, +140, +161, +168, +182, +196, +210, +224, +238, +252,+266, +280, 294, +308, +322, +336, +350, +364 and +378	Days +15, +29, +43, +57, +71, +85, +99, +113, +127, +141, +162, +169, +183, +197, +211, +225, +239, +253, +267, +281, +295, +309, +323, +337, +351 and +365

Subjects and randomisation

Healthy male and female beagle dogs over 4 months in age, with a body weight of between 8 kg and 25 kg and complying with predefined inclusion/exclusion criteria were used. All dogs were dewormed, harboured no ticks at the initiation of the study and were sero-negative for *E. canis* prior to the acclimatisation period. None of the dogs had been treated with a topical or systemic acaricide/insecticide 12 weeks prior to acclimatisation start. Dogs were identified by electronic transponders with unique alphanumeric codes. The study followed a randomised block design and the Day –2 body weight of each dog was used for ranking and group allocation purposes. The 16 dogs initially included were ranked, within gender, in descending order of individual body weights. Animal ID's were used to break ties. Within each gender, animals were then blocked into replicates of two dogs each. Within each replicate, dogs were randomly allocated to groups 1 (untreated group) or 2 (treated group).

Dose and administration of the investigational veterinary product

The investigational veterinary product (IVP) was the imidacloprid 10%/flumethrin 4.5% collar which was fitted according to label instructions on Days 0 and +155 to the dogs in group 2. Dogs in the untreated group were not fitted with placebo collars.

Tick infestation and tick counts

A laboratory-bred *R. sanguineus* tick strain infected with *E. canis* was used for the artificial challenges in the kennel environment. Infectivity of ticks was confirmed by PCR analysis on a sample of between 30–40 *R. sanguineus* ticks taken from each batch of ticks to be used for the artificial challenges. The infectivity of the ticks used for the challenges ranged from approximately 2–17%. For the first tick challenge (on Day +14), 20 ticks were used. But this amount resulted in a low tick burden, so it was decided to increase the number of ticks to 50 to improve the tick challenge. Therefore, from the second challenge (Day +28) onwards, 50 ticks were used. Ticks were released in the sleeping kennels of the dogs and the exact number of ticks used during each challenge was recorded. The ticks used were adult, unfed, at least one week old and had a balanced sex ratio of 1:1 of female and male, respectively. Tick counts were *in situ* on all days except Day +380 when ticks were removed and counted. The findings of the tick counts were categorised as shown in Table 3.

Table 3 Categories of findings from tick counts

Category	General findings	Attachment status
1	Live	Free
2	Live	Attached; unengorged*
3	Live	Attached; engorged**
4	Killed	Free
5	Killed	Attached; unengorged*
6	Killed	Attached; engorged**

* No filling of the alloscutum evident ** Obvious or conspicuous filling of the alloscutum evident

Table 4 Time points for blood and serum collection

Blood collection*	Serum collection
Days +28, +42, +56, +70, +84, +98, +112, +126, +140, +154, +168, +182, +196, +210, +224, +238, +252, +266, +280, +294, +308, +322, +336, +350,+364, +378, +392, +406 and +420	Prior to study inclusion and Days +42, +70, +98, +126, +154, +182, +210, +238, +266, +294, +332, +350, +378 and +406

* Blood was collected from dogs that had not yet been diagnosed and treated for ehrlichiosis and additionally from all dogs with a body temperature of > 39.4 °C for two consecutive days

Blood collection for PCR analysis

Blood was collected from treated and control groups as shown in Table 4. Blood for PCR analysis was collected in EDTA tubes. Blood samples for PCR analysis were tested at ClinVet International (Pty) Ltd. All blood samples taken during scheduled sampling occasions were subject to analysis, but only positive cases are discussed. In cases where clinical signs associated with ehrlichiosis were observed, samples were also taken for PCR and platelet counts only. Total genomic DNA was isolated from whole blood samples using a commercial genomic DNA isolation kit. Polymerase chain reaction entailed the use of primers specific to a region of the *E. canis* dsb gene. Up to 400 ng DNA extracted from whole blood served as template for PCR amplification of the target region. PCR products were analysed using agarose gel electrophoresis. A PCR product of approximately 500 bp indicated the presence of the *E. canis* dsb target region in the sample. Positive, negative, no template, as well as internal amplification controls were included in each run to validate the reactions.

Serum collection for IFA analysis

At least 7 ml of blood was collected from all dogs on days indicated in Table 4. Serum was recovered from the plain tubes, divided into primary and duplicate aliquots and frozen at < –35 °C until assayed for *E. canis* antibodies using a commercial IFA test (IGG IFA, Fuller Laboratory) performed according to the manufacturer's description by the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria, South Africa. All blood samples taken during scheduled sampling occasions were subject to analysis, but only positive cases are discussed.

Dog welfare and monitoring of clinical signs associated with ehrlichiosis

Body temperatures: Rectal body temperatures were recorded for all dogs twice weekly (at least 2 days apart) starting from Day +28 onwards. Rectal body temperatures for dogs with abnormally high values (>39.4 °C) were additionally measured the following day again and if still abnormally high, a clinical examination was conducted. Blood was also collected for platelet counts from these dogs.

Platelet counts: Blood for platelet counts was collected in EDTA tubes on days indicated in Table 4. EDTA blood samples for platelet counts were examined at Pathcare Veterinary Laboratory, Bloemfontein, South Africa.

Clinical examinations: The study dogs were subjected to a clinical examination on the days specified in Table 1. The clinical examination included general appearance by body system, respiration rate, heart rate and body temperature. Additionally, clinical examinations were conducted on all dogs displaying clinical signs associated with CME, such as fever, depression, anorexia, weight loss, haemorrhages, epistaxis and gastrointestinal signs such as diarrhoea. Dogs with abnormally high body temperatures (> 39.4 °C) and dogs with CME-associated clinical signs as observed during the routine daily observations were examined by the veterinarian. To prevent fatal ehrlichiosis, dogs with abnormally high body temperatures (> 39.4 °C) for at least two consecutive days and an abnormally low platelet count were rescue-treated with doxycycline if judged necessary by the examining veterinarian.

General health observations

All animals were observed daily (from Day –7 to +420) for general health conditions and clinical signs of adverse reactions to treatment. Dogs displaying any abnormal signs were examined by a veterinarian.

Statistical analysis

The primary statistical assessment criterion was the number of dogs infected with *E. canis* in the untreated control and the treated group. The secondary statistical assessment criterion was the acaricidal activity of the collar as shown by the number of live ticks collected from the untreated control and the treated group on the various count days.

The level of significance of the formal tests was set at 5% and all tests were two-sided. The groups were compared using an ANOVA with a treatment effect after a logarithmic transformation on the tick (count + 1) data. The proportion of animals infected in each group was compared. SAS Version 8 (Release 8.02 TS Level 02M0) was used for all the statistical analyses.

Calculation of number of dogs infected with *Ehrlichia canis*

A dog successfully infected with *E. canis* was regarded as one that tested serologically positive for *E. canis* antibodies (IFA essay) and tested positive for the presence of *E. canis* DNA by PCR analysis.

Any dog that complied with these two criteria was regarded as successfully infected.

The percentage blocking efficacy for the treatment group was calculated as follows:

Efficacy (%) = 100 x (Tc – Tt) / Tc

Tc = Total number of infected dogs in the negative control group 1

Tt = Total number of infected dogs in the treatment group 2

Calculation of acaricidal activity of the collar

Efficacy against ticks was calculated for the treatment group at each assessment day according to the formula given below. Due to the fact that small and even zero tick counts were recorded, it was expected that the tick counts would not follow a normal distribution. Therefore, it was decided that the primary efficacy calculations would be based on geometric means rather than arithmetic means. The calculations were based on the geometric means of the tick (count + 1) data. One (1) was subsequently subtracted from the result to obtain a meaningful value for the geometric mean of each treatment group. Efficacy calculations based on arithmetic means were, however, also reported.

Percent efficacy against ticks was calculated as follows:

Efficacy (%) = 100 x (Gmc – Gmt) / Gmc

Gmc = Geometric or arithmetic mean number of live ticks (categories 1–3, as explained in Table 3) on dogs in the negative control group (group 1) at a specific time point

Gmt = Geometric or arithmetic mean number of live ticks and dead ticks (categories 1–3 and 6) in the treatment group (group 2) at a specific time point

Results

PCR Results

Ehrlichia canis DNA was detected in four collar-treated dogs of which an infection could only be confirmed in two of these dogs by IFA assay. *E. canis* DNA was detected in 36 out of the 39 untreated control dogs throughout the study experimental phase.

IFA results

Up to Day +350, no *E. canis* antibodies were detected in any of the collar-treated dogs. Sero-conversion was, however, observed in three collar-treated dogs on Day +378 and in two dogs on Day +406. During the course of the experimental phase, 39 untreated control dogs were exposed to the environmental tick challenge of which only one dog did not sero-convert by Day +406.

Ehrlichia canis blocking efficacy

Infections with *E. canis* were confirmed by IFA and PCR in 2 collar-treated dogs and 36 untreated dogs over the 420 day assessment period. Infection with *E. canis* was, however, only first confirmed in the two collar dogs on Day +378. The IVP collar, therefore, reduced the risk of infections by 100% up to Day +378 and by 94.4% up to Day +420.

Tick count results

Low tick burdens were observed on the dogs during the *in situ* tick counts on Day +14, so the number of ticks used for subsequent challenges was increased from 20 to 50. This change was assessed as having no negative impact on the study and improved the tick challenges on dogs.

Arithmetic and geometric mean tick counts on the various assessment days for the two study groups are summarised in Table 5. The arithmetic mean tick counts recorded for the negative control group ranged from 1.5 to 29.0. The geometric mean tick counts recorded for the treatment groups differed statistically significantly ($p<0.05$) from that of the untreated control group (group 1) on all assessment days.

Acaricidal activity results

Efficacy values of acaricidal activity (%) based on arithmetic and geometric mean tick counts are summarised in Table 5. Efficacies based on geometric means were considered primary. The IVP collar was highly effective against *R. sanguineus* ticks in a simulated kennel environment with efficacies ranging from 90.0–100% for the duration of the assessment period.

Dog welfare and clinical signs associated with ehrlichiosis discussion

Of the control and treated groups totalling 39 and 8 dogs, respectively, 28 control dogs and two treated dogs had to be rescue-treated for ehrlichiosis. All dogs recovered completely.

Body temperatures

The body temperature ranges recorded during the routine measurements (excluding those conducted during clinical examinations) for the dogs included in the study experimental phase are summarised in Table 6. Elevated body temperatures (>39.4 °C) were observed in two collar-treated dogs and 25 of the 39 untreated control dogs exposed to the environmental tick challenges. Infection with *E. canis* was confirmed in all dogs with elevated body temperatures.

Platelet counts

The platelet counts are summarised for all dogs in Table 6. Low platelet counts were observed an all but two dogs confirmed infected with *E. canis*.

Clinical examinations

The abnormal clinical signs generally associated with ehrlichiosis observed during the clinical observation conducted at a minimum every two weeks are summarised in Table 7. The most common abnormal clinical signs observed were enlarged superficial, submandibular and popliteal lymph nodes.

Table 5 Tick count results (as arithmetic and geometric means of groups 1 and group 2) and percentage efficacy values (as calculated from both arithmetic and geometric means)

Day	Group 1 (Untreated)		Group 2 (Collar)		Efficacy (%)	
	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean
15	1.5	1.4	0.0	0.0	100.0	100.0
29	4.6	3.7	0.0	0.0	100.0	100.0
43	5.3	4.9	0.0	0.0	100.0	100.0
57	10.2	9.1	0.0	0.0	100.0	100.0
71	7.0	6.5	0.0	0.0	100.0	100.0
85	9.3	5.8	0.0	0.0	100.0	100.0
99	9.0	7.3	0.0	0.0	100.0	100.0
113	13.4	11.5	0.0	0.0	100.0	100.0
127	15.3	13.7	0.0	0.0	100.0	100.0
141	10.9	9.6	0.0	0.0	100.0	100.0
162	9.0	8.1	0.1	0.1	98.6	98.9
169	15.9	12.6	0.1	0.1	99.2	99.3
183	13.4	11.6	0.1	0.1	99.1	99.2
197	13.6	12.9	0.1	0.1	99.1	99.3
211	8.0	7.1	0.1	0.1	98.4	98.7
225	7.1	5.4	0.3	0.1	96.5	97.3
239	8.9	7.9	0.0	0.0	100.0	100.0
253	11.3	10.8	0.3	0.2	97.8	98.2
267	17.4	16.7	0.8	0.5	95.7	97.1
281	16.8	15.9	0.5	0.2	97.0	98.6
295	14.4	13.1	0.4	0.3	97.4	97.7
309	17.3	16.3	2.1	1.5	87.7	90.9
323	11.3	8.2	0.4	0.3	96.7	96.9
337	21.0	19.9	0.3	0.2	98.8	99.1
351	26.0	23.2	0.0	0.0	100.0	100.0
365	29.0	29.0	1.6	0.6	94.4	98.1
380	17.3	10.6	0.0	0.0	100.0	100.0

General health observations

The signs recorded from dogs during the daily general health observations are summarised in Table 8. All of the signs were seen in group 1 dogs and are associated with ehrlichiosis. Dogs received appropriate veterinary care.

Adverse events

No adverse events occurred during the clinical examinations and daily health observations which were regarded as being related to the administration of the collar.

Table 6 Body temperatures, platelet counts, PCR and IFA analyses results

ID	Group	Day		*E. canis* DNA detected by PCR (Day)	Antibodies detected by IFA (Day)	Gender	Body temp. range (°C)		Days temp. > 39.4 °C	Platelet count x 10^9/l		Days platelet count out of range (200–500 x 10^9/l)
		Inclu–ded	Exclu–ded				Min	Max		Min	Max	
CBD FC0	2	–2	+420	+70, +378,+390, +392	+378, +406	M	37.8	39.9	+386, +390	4	409	+390, +392
CBF BBA	2	–2	+420	–	–	M	37.5	39.0	–	203	371	–
CD5 02D	2	–2	+420	–	+378	M	37.6	38.8	–	278	472	–
CC0 F72	2	–2	+420	–	–	F	37.8	39.2	–	282	541	+378
CD4 621	2	–2	+420	+70	–	F	37.0	38.7	–	21	403	+28, +208, +210
CD3 440	2	–2	+420	–	–	M	37.2	38.6	–	226	436	–
957 4F4	2	–2	+420	+378, +392, +406	+378, +406	M	37.5	39.7	+393	3	361	+392, +406
CD2 370	2	–2	+420	+224	–	F	37.3	38.8	–	274	510	+42
CD1 665	1	–2	+166	+140, +146, +154	+154	M	38.0	39.7	+145, +146	3	336	+146
CC1 C33	1	–2	+107	+70, +85	+98	M	38.3	39.6	+85	24	587	+35, +98
CD3 025	1	–2	+57	+42, +47, +49, +56	+42	M	38.2	39.9	+47, +48, +50	12	341	+48
CD4 7AE	1	–2	+218	+196, +208, +210	+210	M	37.7	39.6	+208	1	374	+208, +210
CC1 C42	1	–2	+79	+55, +56	+70	F	37.6	39.6	+54	2	691	+28, +35, +55, +56, +70, +112
CC1 315	1	–2	+57	+28, +28, +40 +42	+42	F	37.7	39.7	+36	4	478	+36, +42
B8B D46	1	–2	+107	+85	+98	F	37.5	39.9	+85	137	630	+42, +56, +35, +98
6DC 659	1	–2	+79	+70, +76	+70	F	37.3	39.9	–	9	434	+70, +76
436 B01	1	+59	+138	+112, +116, +124	+126	M	37.1	39.1	–	3	361	+124, +126
9B5 23D	1	+59	+168	+140, +149, +154	+154	M	37.6	40.7	+148	40	386	+148
E9C 096	1	+97	+107	+98, +104	+107	F	37.9	40.0	+104, +105, +106	2	346	+104
CC1 242	1	+79	+420	+168, +378	+378, +406	F	37.8	40.2	+379, +380	53	621	+98, +126, +140, +154, +308, +392
E16 D69	1	+107	+251	+154, +196	+238	F	37.5	39.3	–	3	569	+126, +140, +182, +182, +201, +224, +196
DF4 B1B	1	+107	+218	+126, +212	+182	F	38.2	39.2	–	8	356	+210
DF4 E09	1	+107	+168	+112, +126, +131, +140	+126	F	38.2	39.4	–	2	616	+129, +140

Table 6 (continued) Body temperatures, platelet counts, PCR and IFA analyses results

ID	Group	Day		*E. canis* DNA detected by PCR (Day)	Antibodies detected by IFA (Day)	Gender	Body temp. range (°C)		Days temp. > 39.4 °C	Platelet count x 10^9/l		Days platelet count out of range (200–500 x 10^9/l)
		Inclu–ded	Exclu–ded				Min	Max		Min	Max	
CC1 369	1	+138	+218	+182, +184	+182	F	36.9	39.1	–	305	344	–
B8D 7FE	1	+166	+218	+182, +196	+210	M	38.1	38.9	–	38	329	+196
EA1 535	1	+166	+218	+196	+238	M	37.9	38.6	–	143	256	+168
CDE 2A2	1	+166	+218	+196, +201	+210	M	37.6	39.1	–	38	467	+201
E18 3E8	1	+188	+218	+208, +210	+210	F	38.2	40.6	+208	4	361	+208, +210
E17 BFF	1	+218	+251	+238, +241	+238	M	38.0	40.3	+239, +243	5	415	+238, +240
E4B 117	1	+218	+251	+238, +241	+238	M	38.3	39.9	+243	1	361	+208, +210, +238, +240
EA0 FF6	1	+218	+293	+238, +254, +266	+266	F	37.4	38.7	–	64	544	+224, +238, +252, +280
DF6 FC9	1	+218	+331	+238	+294	F	37.6	39.0	–	243	325	–
E9B E23	1	+218	+251	+238, +241	+238	M	38.2	40.6	+243	1	374	+238, +240
CC5 FB7	1	+218	+251	+238, +241	+238	F	38.0	41.2	+243	42	327	+238, +240
CD0 108	1	+251	+321	+226, +279, +280	+294	M	37.2	39.9	+271, +274	0	360	+266, +276, +280
DF7 72D	1	+251	+331	–	+238, +266	F	37.4	38.4	–	270	337	–
6E1 208	1	+251	+371	+308, +316, +322, +336,+350,+364	+322, +350	F	36.8	39.6	+316	0	525	+280, +316, +322, +336, +364
DF7 8A3	1	+251	+371	+308, +316, +322, +364	+322, +350	F	37.4	40.2	+316, +323	1	363	+316, +322, +350, +364
E9F F45	1	+251	+420	+350, +358, +364, +402	+378, +406	M	37.7	39.6	+358, +359	20	322	+359, +420
B8C B85	1	+293	+371	+308, +322, +323, +336, +350, +364	+322, +350	F	37.6	39.5	+320	3	459	+322, +336, +364
6DF CED	1	+321	+371	+350, +364	+350	F	37.5	40.3	+351, +354, +356	2	538	+336, +350
CBE DE2	1	+33	+420	+350, +356, +364	+378, +402	F	38.2	39.9	+355, +356, +358, +359	29	387	+350, +357, +364
DF6 BEF	1	+331	+420	+350, +358, +364	+378, +402	F	38.0	40.2	+358, +359	5	364	+359, +364
DF8 A94	1	+371	+420	–	–	F	37.8	38.6	–	331	409	–
EA0 CA1	1	+371	+420	–	+378	M	38.0	38.9	–	294	435	–
DF7 E5C	1	+371	+420	+392, +399, +406	+406	M	38.3	40.1	+397, +399, +400	18	362	+378, +392
E9C 581	1	+371	+420	–+392, +406, +420	+406	M	38.1	39.5	+386	124	368	+392, +406, +420

Table 7 Clinical examination summary (only animals for which abnormal signs were recorded are tabulated)

ID	GR	*E. canis* DNA detected by PCR (Day)	Clinical observation	Study day(s)
CBD FC0	2	+70, +378,+390, +392	Lymph nodes enlarged/swollen	+252
CBF BBA	2	–	Lymph nodes enlarged/swollen	+224, +238, +280, +406
CD4 621	2	–	Superficial lymph nodes enlarged	+224
CD3 440	2	–	Lymph nodes enlarged/swollen	+266
957 4F4	2	+378, +392, +406	Lymph nodes enlarged/swollen	+266, +392
CD2 370	2	–	Lymph nodes enlarged/swollen	+224, +266, +322, +331
CD4 7AE	1	+196, +208, +210	Habitus abnormal; still, dull	+210
6DC 659	1	+70, +76	Lymph nodes enlarged/swollen	+76
CC1 242	1	+168, +378	Lymph nodes enlarged/swollen	+224, +378
E16 D69	1	+154, +196	Lymph nodes enlarged/swollen; habitus abnormal	+224
E18 3E8	1	+208, +210	Habitus abnormal; still, dull	+210
DF6 FC9	1	–	Lymph nodes enlarged/swollen	+224, +238, +252, +280, +322
E9B E23	1	+238, +241	Lymph nodes enlarged/swollen	+224, +238
CD0 108	1	+226, +279, +280	Lymph nodes enlarged/swollen	+252, +280
			Serous nasal discharge	+280
DF7 72D	1	–	Lymph nodes enlarged/swollen	+322
6E1 208	1	+308, +316, +322, +336,+350,+364	Lymph nodes enlarged/swollen	+322, +331, +350, +364
DF7 8A3	1	+308, +316, +322, +364	Lymph nodes enlarged/swollen; dull	+322
			Lymph nodes enlarged/swollen; tick bite dermatitis	+331
			Lymph nodes enlarged/swollen	+350, +364
E9F F45	1	+350, +358, +364, +402	Lymph nodes enlarged/swollen	+331
B8C B85	1	+308, +322, +323, +336, +350, +364	Lymph nodes enlarged/swollen	+322
CBE DE2	1	+350, +356, +364	Lymph nodes enlarged/swollen	+364
DF6 BEF	1	+350, +358, +364	Lymph nodes enlarged/swollen	+331, +364

ID = Idenification; GR = Group; PCR = Polymerase chain reaction; G = Gender.

Discussion and conclusion

The study reported here was conducted in the spirit of the VICH GL9 (July 2000) guideline on good clinical practice to assure high quality standards. The study was controlled and the animals were randomised. The control group animals were not fitted with placebo collars, so blinding was not possible, but the possibility of bias was considered to be minimal as IFA, PCR and tick counts are objective standardised procedures.

Table 8 General health observation summary

Animal ID	Group	Day	Observation
CC1 315	1	+36	Listless
CC1 315	1	+38	Listless
6DC 659	1	+76	Depresses
6DC 659	1	+77	Listless
436 B01	1	+124	Listless
436 B01	1	+125	Poor appetite
436 B01	1	+126	Listless and poor appetite
9B5 23D	1	+148	Listless
CC1 369	1	+188	Listless (Body temp. 39.6 °C)
E16 D69, CDE 2A2	1	+201	Listless, poor appetite
CD4 7AE	1	+208	Poor appetite
CD0 108	1	+278	Listless
6E1 208	1	+316	Poor appetite, listless

Also the 2% to 17% prevalence of *E. canis* infection in the ticks used for the challenges was regarded as representative for field situations, as prevalences of *E. canis* in ticks reported from different endemic areas (either mammalian hosts or questing adults in the environment) vary between 0.09% and 10% (Aguiar et al. 2007; Satta et al. 2011; Harrus et al. 2011).

The primary objective was the transmission prevention ability for *E. canis* and, therefore, the number of dogs infected by *E. canis*. None of the Seresto® collar-treated dogs were infected for the first year of the study, despite being subjected to 216 infestation events (= animals x infestation time points). Towards the end of the experimental phase (Day +378), two collar-treated dogs were infected by *E. canis* as confirmed by PCR and IFA. By contrast, dogs in the control group had to be replaced frequently due to *E. canis* infections, necessitating a total of 39 dogs for the complete experimental phase. Of these 39, 36 became infected as confirmed by IFA and PCR.

The secondary objective was the acaricidal efficacy based on tick counts. The geometric mean tick count for the collar-treated dogs was low and ranged from 0.0 (at the first ten time points) to 1.5 during the entire assessment period. In comparison, the geometric mean tick count for untreated dogs was considerably higher and ranged from 1.4 (at the first time point when only 20 ticks were used to challenge) to 29.0 during the same period. Acaricidal efficacies ranged from 90.0% to 100% for the entire assessment period.

In summary, the empirical efficacy of the Seresto® collar in preventing long-term transmission of *E.canis* by infected *R. sanguineus* ticks to dogs has been evaluated for the first time. The collar prevented *E. canis* transmission by 100% up to Day +378 (197 days after the most recent collar application) and by 94.4% up to Day +420 (223 days after the most recent collar application). Acaricidal efficacy ranged from 90.0% to 100%. These results show that the collar is not only effective at killing *R. sanguineus,* but also that the onset of acaricidal action is quick enough to prevent ticks from transmitting the *E. canis* infection over a time period consistent with the label efficacy period of 8 months.

Ethical standards

All institutional and national guidelines for the care and use of laboratory and study animals were followed.

Conflict of interest

This clinical study was completely funded by Bayer Animal Health GmbH, of which D Stanneck (Germany) is an employee. ClinVet, of which JJ Fourie is an employee, is an independent, South African, Contract Research Organisation contracted to manage the conduct of the study.

Acknowledgements

The authors acknowledge the contributions of technical staff at ClinVet International (Pty) Ltd (Republic of South Africa) in executing the study and ensuring that Good Scientific Practice standards were adhered to.

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