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Original Article

Evaluation of the Persistent Preventive Efficacy of 2.5% Moxidectin/10% Imidacloprid Spot-on (Advocate®, Advantage® Multi) in Dogs Experimentally Infected with *Angiostrongylus vasorum*

Claudia Böhm[1] ✉, Gabriele Petry[1], Holger Schmidt[2], Katharina Raue[3], Franziska Barthel[1], Roland Schaper[1]

[1] Bayer Animal Health GmbH, 51368 Leverkusen, Germany
[2] BioMedVet Research GmbH, 29664 Walsrode, Germany
[3] Institute for Parasitology, University of Veterinary Medicine, 30559 Hannover, Germany

Corresponding author:
Dr. Claudia Böhm
✉ E-mail: claudia.boehm@bayer.com

Abstract

A controlled, randomized and blinded dose-confirmation study was conducted to assess the persistent preventive efficacy of treatment with Advocate® spot-on (2.5% moxidectin/10% imidacloprid) in dogs four weeks before inoculation of third-stage larvae of *Angiostrongylus (A.) vasorum*. Twenty-four adult dogs were randomly allocated to three treatment groups. Dogs in group 1 were treated with Advocate® spot-on at the minimum recommended dose of 0.1 mL per kg bodyweight (BW) on day 84 and inoculated with third-stage larvae of *A. vasorum* on day 112. Dogs in group 2 were treated monthly with Advocate® spot-on on days 0, 28, 56 and 84 at the minimum recommended dose of 0.1 mL per kg BW and inoculated with third-stage larvae on day 112, i.e. 28 days after the last treatment. Dogs in the third group served as inoculated but untreated controls. All dogs were euthanized and necropsied 70–72 days after experimental inoculation. No dogs in group 1 or group 2 had any immature or adult *A. vasorum* worms detected at the post mortem examination, whereas adult worms (and in some cases also immature worms) were recovered from all dogs in the untreated control group. The study results demonstrate that a single Advocate® administration has persistent efficacy for one month, protecting dogs against infection with *A. vasorum* and thus preventing the lung damage caused by immature adults. In endemic areas, monthly treatment of dogs with Advocate® spot-on will protect the dog from lung damage associated with early

stages of *A. vasorum* and from patent infection with *A. vasorum*. This first report on preventive efficacy significantly adds to knowledge and possibilities veterinarians have to prevent disease.

Introduction

A. vasorum, the "French heartworm" has an indirect life cycle with snails and slugs as intermediate hosts and canids as final hosts. The parasite is widespread in Europe and Canada, but is also observed in other parts of the world, indicating a cosmopolitan distribution covering Europe, North and South America (Ferdushy and Tabarak 2010, Elsheikea et al. 2014). Infected dogs are difficult to identify, as the clinical signs are unspecific (Willesen et al. 2007). The Baermann migration-sedimentation technique to detect first-stage larvae (L1) of *A. vasorum* in faeces is labour-intensive and of low sensitivity. The diagnosis of serum antigen using a newly developed ELISA is a more specific and more sensitive technique for *A. vasorum* (Schnyder et al. 2011). A commercial antigen test for dog serum is now also available (AngioDetectTM, IDEXX Laboratories, Ludwigsburg, Germany). Epidemiological surveys indicate that the prevalence of *A. vasorum* infection in dogs and foxes is increasing in various European countries (Traversa et al. 2013, Taylor et al. 2015). A significant increase in the prevalence of *A. vasorum* from 0.9% in 2004–2006 to 1.8% in 2012–2016 was found in a retrospective analysis of canine faecal samples from Germany (Barutzki et al. 2017). In addition, there are new reports on the occurrence of *A. vasorum* infections in dogs and wildlife from countries where *A. vasorum* was previously not observed (Hurniková et al. 2013, Lempereur et al. 2016, Penagos et al. 2016).

After ingestion of a snail or slug containing third-stage larvae (L3) of *A. vasorum*, or even ingestion of grass or dog food contaminated with the slime of an infected gastropod (Ferdushy and Tabarak 2010, Conboy et al. 2017), the L3 penetrates the intestinal wall and migrates to the abdominal lymph nodes, where it moults into fourth-stage larvae (L4) and immature adults. Immature adults migrate to the right heart and to the pulmonary arteries, where they become mature. The prepatent period may vary from 38 to 75 days. The eggs are transported via the blood stream to the pulmonary capillaries, where L1 are released. These L1 penetrate the capillary and the alveolar wall and migrate upwards in the respiratory tract. Subsequently they are swallowed and shed via faeces to infect the intermediate host, i.e. a snail or slug. Clinical signs of angiostrongylosis in dogs can be serious and may lead to death (Traversa et al. 2013, Bolt et al. 1994). In a study with experimentally infected dogs it was demonstrated that even the immature adults cause lung tissue damage (Schnyder et al. 2009).

Advocate® spot-on is a broad-spectrum antiparasitic for dogs. It is approved for treatment of various ectoparasite and endoparasite species. For some major gastrointestinal nematodes, in addition to the treatment of patent infections, the product is also efficacious against L4 and immature adults. Efficacy has also been confirmed against larval stages of *Dirofilaria immitis* and *Dirofilaria repens* (EMA 2017a). The efficacy of Advocate® spot-on against *Angiostrongylus vasorum* in naturally infected dogs was demonstrated by Willesen et al. (2007). Schnyder et al. (2009) experimentally inoculated dogs with L3 of *A. vasorum*. Eight dogs each were treated with Advocate® spot-on at 4 and 32 days post infection. None of the treated dogs developed a patent infection, with no worms being detected in any of the dogs at necropsy on days 56–59. Advocate was shown to be 100% effective against L4 and immature adults of *A. vasorum*. Schnyder et al. (2009) also demonstrated that early treatment on day 4 post infection (to kill L4 larvae) prevented the development of tissue damage in the lung of the dogs, whereas in the group where development to the immature adult stage was allowed to take place, disseminated remnants of granulomatous inflammation could be detected by histological examination of lung tissue in seven of eight of the dogs which went on to be successfully treated

32 days post infection. Arterial thrombi were also found in four of these eight dogs. This study demonstrated that only the earlier treatment post inoculation of L3 was able to completely prevent lung damage caused by immature adults of *A. vasorum*. The objective of the present study was to assess the ability of the persistent efficacy of Advocate® over 4 weeks to prevent patent *A. vasorum* infections in dogs and early damage in lung tissue caused by the development of immature adults.

Materials and methods

Study design

The study was conducted as a controlled, randomized, blinded dose-confirmation study in accordance with VICH guideline 9 "Good Clinical Practice" (July 2001) and the recommendations given in VICH Guideline 7 "Efficacy of anthelmintics: General requirements" (December 2000), VICH Guideline 19 "Efficacy of anthelmintics: Specific Recommendations for Canines" (July 2002), the WAAVP guidelines for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) and European animal welfare requirements. For the study, 24 dogs aged eight to nine months were used, both male and female. The dogs were fed commercial dog food and had access to water ad libitum. Three months before the start of the study the dogs were not treated with any macrocyclic lactones or other drugs with anthelmintic efficacy. Dogs were acclimatized to the study facility before the study start for 11 days. Within the acclimatization time between study day (SD) -11 and SD -2 faecal samples were taken on three consecutive days to determine the status of pre-existing gastrointestinal nematodes and/or lungworm infection. Sedimentation/flotation and the Baermann technique were used to show the presence of any nematode eggs, larvae or adult worms. All dogs underwent physical examinations on SD -11, SD -1, SD 112 and on the day of necropsy before euthanasia. Dogs were weighed on defined study days (-1, 27, 55, 83, 112, 178 and 182–184) to calculate the Advocate® doses for treatment, the doses of sedative administered prior to inoculation of infective material and the doses of pentobarbital administered for euthanasia. Individual faecal samples were taken on SD 171, 174 and 176 to detect the start of patency in all individual dogs using the Baermann technique.

Treatment protocol

On SD -1 all 24 dogs were randomly allocated to three groups of eight dogs each (four males and four females). Dogs in group 1 were treated with Advocate® spot-on at the minimum recommended dose of 0.1 mL per kg BW corresponding to 2.5 mg moxidectin/kg and 10 mg imidacloprid/kg BW at day 84 and infected with L3 of *A. vasorum* on day 112, i.e. 28 days after treatment. Dogs in group 2 were treated monthly with Advocate® spot-on on SD 0, 28, 56 and 84 at the minimum recommended dose of 0.1 mL per kg BW and infected with L3 on SD 112, i.e. 28 days after the last treatment. Dogs in group 3 served as infected but untreated controls (Table 1).

Moxidectin, the active ingredient in Advocate® that is effective against nematodes, is quickly absorbed

Table 1 Study design and allocation to treatment

Study group	No. of animals	Treatment	Frequency of application	Inoculation of 250 L3 larvae of *A. vasorum*
1	8	Advocate® spot-on	single application at SD 84	SD 112
2	8	Advocate® spot-on	4 times at monthly intervals (SD 0, 28, 56, 84)	SD 112
3	8	none	---	SD 112

through the skin and reaches maximum plasma concentrations 4–9 days after application. Significant concentrations persist for at least 28 days, and subsequent treatment administered at 4-weekly intervals will lead to a steady state level after 4 repeated doses (EMA 2017b and Bowman et al. 2016).

Under natural conditions, trickle infection of dogs with *A. vasorum* L3 between two monthly Advocate® treatments may lead to a potential continuous uptake of L3 until the next treatment, and an effective level of moxidectin for 4 weeks is therefore essential to prevent the development of larval and immature adult stages and the resulting damage to lung tissue.

Experimental inoculation of dogs

The inoculum was prepared at the University of Veterinary Medicine Hannover (Institute for Parasitology). Snails (*Achatina fulica*) were infected with about 3000 L1 of *A. vasorum*. The *A. vasorum* originated from infected foxes in Denmark. A technique described by Schnyder et al. (2009) was used to extract *A. vasorum* larvae from snails. Infected snails were detached from their shell and cut into small pieces. Afterwards the mass was minced and mixed with 150 mL digestion solution (10 g pepsin and 10 mL HCl 37% in 1 L water). The mixture was stirred at 43°C for 35 minutes. Subsequently the digesta were sieved (mesh sized 220 µm) and stored at 30°C for 30 minutes to let the larvae sediment. The supernatant was removed by centrifugation. The remaining sediment was refilled with tap water and once again centrifuged. This washing was repeated twice. Afterwards the number of L3 per aliquot was counted and adjusted to approx. 250 L3 per inoculum. Prior to inoculation dogs were sedated with propofol (Narcofol®, 0.6 mL/kg) followed by metoclopramide (Metomotyl®, 1 mL/dog) to avoid regurgitation of the inoculum. As soon as the depth of anaesthesia was sufficient, a stomach tube was inserted and the inoculum was given directly into the stomach. Afterwards water was used to rinse the tube before removing it. All dogs were kept sedated and observed for the first hour after inoculation to exclude vomiting.

Worm counts at necropsy and post mortem examination

All dogs were euthanized and necropsied between SD 182 and SD 184. Dogs first received heparin sodium 350 IU/kg BW and three minutes later pentobarbital 1 mL/kg BW. Afterwards the thorax was opened, vessels from the heart and lungs were clamped. The worm burden was determined by a reverse lung perfusion technique (Schnyder et al. 2009). In the left heart ventricle isotonic saline solution was pumped through the pulmonary veins, pulmonary capillaries and pulmonary arteries to the pulmonary trunk. The mixture of blood and isotonic saline solution was then drained from the pulmonary trunk into flasks. After the rinsing procedure, the heart and lung were extracted from the thorax. The heart was completely opened, the lumen was rinsed with isotonic saline solution over a fine sieve (100 µm). Trachea and bronchi were opened longitudinally, the lung was cut into very small pieces (0.5 mm) and visible worms were collected and transferred into vessels containing isotonic saline solution. The little pieces of lung were afterwards rinsed thoroughly over a fine sieve (100 µm) with tap water with the aim of collecting further worms. The worms from each dog were collected in tubes with 50 mL capacity. The mixture of blood and isotonic saline solution was also sieved (100 µm) and visible worms were collected in petri dishes.

All worms including ones that had recently died and worm pieces with intact integument were counted as viable worms. All worms which were obviously dead before necropsy started (i.e. lytic, necrotic worms with obvious integument changes) were counted as dead. Each head or tail was also counted, in which case the higher number was referred to. Larval stages and immature adults were also listed. Macroscopic changes in the lungs were recorded but not quantitatively assessed.

Efficacy determination and statistical analysis

The adequacy of infection was determined according to VICH guidelines 9 and 19. These require a minimum of six animals in the control group with at least five worms each.

The primary efficacy criterion in response to treatment with Advocate® spot-on was the number of live adult *A. vasorum* at necropsy; this was calculated according to VICH guideline 7 and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) as follows:

$$\%\ \text{efficacy} = \frac{\text{geomean worm count (untreated)} - \text{geomean worm count (treated)}}{\text{geomean worm count (untreated)}} \times 100$$

Results

All dogs were free of gastrointestinal worms and lungworms at study inclusion. After inoculation on SD 112, faecal larval counts on SD 171, 174 and 176 to detect the start of patency were negative for L1 of *A. vasorum* in all dogs in groups 1 and 2. In group 3, in all eight dogs L1 were observed in faecal samples taken on day 171 and day 176 and in six of eight dogs in faecal samples taken on day 174.

At necropsy no dogs in groups 1 and 2 had any macroscopic changes in the lungs. In seven of eight dogs in the untreated control group lungs were mildly to severely affected, firm nodules, haemorrhagic lesions and atelectatic areas were recorded.

Efficacy evaluation

The primary efficacy criterion in response to treatment with Advocate® spot-on was the number of live adult *A. vasorum* worms at necropsy. No adult worms and no larval stages/immature adults of *A. vasorum* were found in any of the dogs in group 1 and 2. All eight dogs in group 3 harboured adult worms (Table 2). All worms found were judged viable.

The geometric mean of total worm counts in dogs in the untreated group was 56.7. Hence, the requirements for the adequacy of infection with *A. vasorum* were fulfilled. No worms could be isolated from dogs in group 1 and 2. Based on the geometric means of live worm counts, the efficacy was 100 % in both Advocate®-treated groups 1 and 2 ($p < 0.0001$).

Table 2 Number of worms and immature adults at necropsy in dogs in group 3 (untreated, necropsy on days 70 to 72 post experimental inoculation with L3 of *A. vasorum*)

Dog ID	Blood male	Blood female	Heart male	Heart female	Lung male	Lung female	Total male	Total female	Total m + f	Immature adults
392	21	28	3	2	7	10	31	40	71	0
1402	16	15	6	6	0	6	22	27	49	1
1559	9	10	0	1	1	2	10	13	23	7
1577	20	33	2	4	7	9	29	46	75	0
1598	28	21	4	9	6	11	38	41	79	0
9423	17	55	0	2	13	12	30	69	99	0
9520	0	3	4	9	3	6	7	18	25	3
9557	36	12	2	4	18	19	56	35	91	10
geomean	13.1	17.0	2.0	3.9	4.6	8.2	23.4	32.2	56.7	2.6

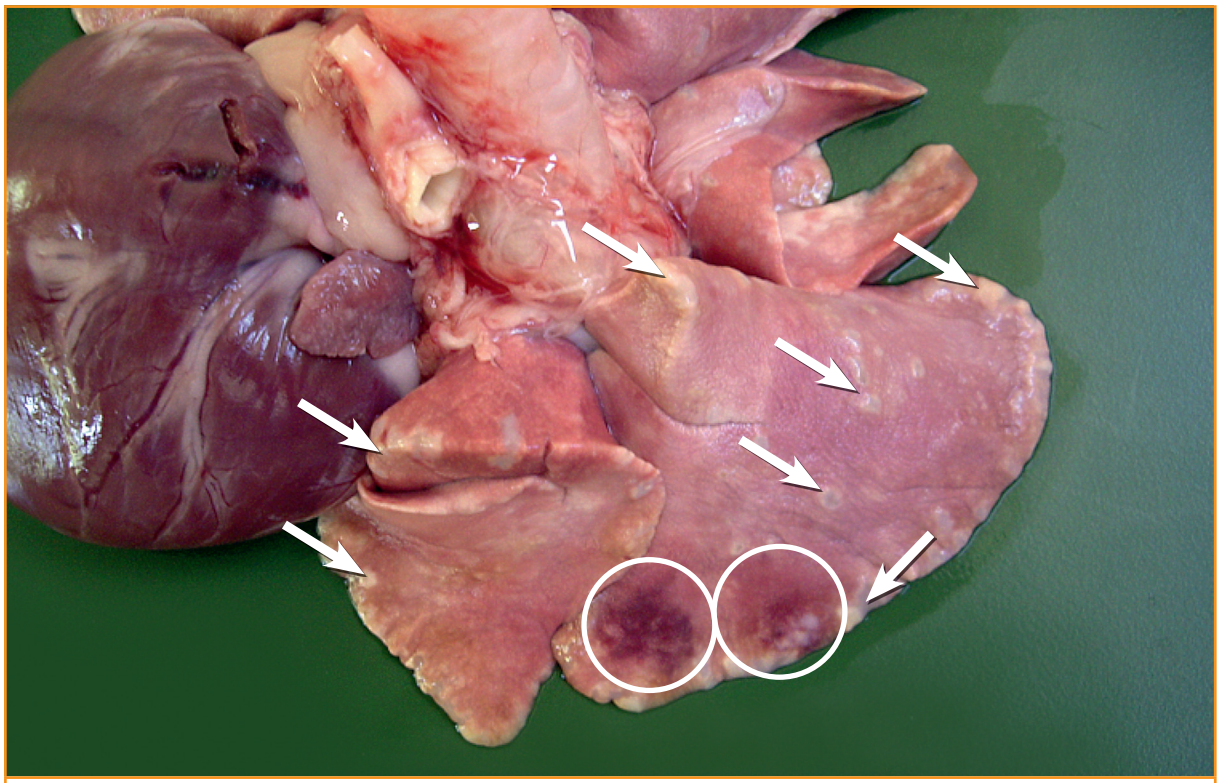

Fig. 1 Pathological lesions of a lung from a dog inoculated with 200 third-stage larvae of *Angiostrongylus vasorum* and 32 days allowed for development of immature adult stage before being given successful treatment with imidacloprid/moxidectin spot-on solution (Advocate®). Necropsy was done on day 56 (Schnyder et al. 2009).
Lung lobes show whitish granulomas (arrows) and hyperemic areas (circles) especially at the margins of the lobes.

Health observations

Treatment with imidacloprid/moxidectin spot-on was well tolerated by all dogs. No relevant clinical signs of intolerance were observed beside one dog who vomited once after first application. The whole study population stayed clinically healthy throughout the study period.

Discussion

The present study was conducted according to VICH good clinical practice, which assures the accuracy, integrity and correctness of the observations recorded. The study was controlled by an untreated control group, dogs were randomized to groups and the scientists collecting the study results were blinded.

The objective of the study was to prove the persistent efficacy of an Advocate® spot-on treatment against L3/L4/immature adults in preventing establishment of *A. vasorum* in dogs and protecting lung damage caused by immature adults (Fig. 1). Advocate® spot-on has already demonstrated persistent efficacy against *Dirofilaria repens* and *Dirofilaria immitis*. A single Advocate® spot-on treatment will provide efficient protection against L3 of *D. repens* for four weeks, thus preventing the development of stages that affect the dog's health (Genchi et al. 2013).

For *D. immitis* Bowman et al. (2016) demonstrated a steady state serum level of moxidectin after four Advocate® spot-on applications at monthly intervals. Subsequently, i.e. four weeks after the last treatment, the authors infected dogs with *D. immitis* L3 subcutaneously. The persistence of

the L3 efficacy of the steady state serum concentration of moxidectin was confirmed by the absence of adult heartworms in the treated dogs at necropsy on day 152 after infection.

Furthermore, Bowman et al. (2017) demonstrated that even a single application of 10 % imidacloprid + 2.5 % moxidectin topical solution leads to 100 % prevention of infection with *D. immitis* L3. Following a single treatment with Advocate®, experimental infection with 50 L3 of *D. immitis* was performed 30 days post treatment. When necropsy was done 148 days following infection, no worms were recovered from the treated group, whereas the control group harboured a range of 2–33 worms in six of the eight dogs. 100% prevention of infection for 30 days following a single application was therefore demonstrated.

It was considered likely that Advocate® spot-on would also be efficient against larval stages of *A. vasorum* during four weeks after application. For *A. vasorum* the elimination of larval stages is of importance not only to prevent the development of mature nematodes but also to prevent the damage in lung tissue already caused by immature adults within the first four weeks after infection. Follow-up treatment to maintain efficacious plasma levels of moxidectin throughout the entire four week period is important in the prevention of disease caused by immature adults between two monthly treatments.

The present study tested two treatment regimens to reach this final objective. In study group 1 the efficacy against L3/L4 of a single application of Advocate® spot-on was tested. Dogs were inoculated with L3 of *A. vasorum* four weeks post treatment. By this time the moxidectin serum level should already have declined from the Cmax, which is reached at four to nine days post treatment. In dogs in group 2 Advocate® spot-on was applied four times at four week intervals before dogs were inoculated with L3 of *A. vasorum* four weeks later. This treatment regimen investigated the influence of increased moxidectin serum levels after reaching steady state.

In dogs in both treatment groups the preventive efficacy against L3/L4 of *A. vasorum* was 100 %. This was independent of the number of preceding Advocate® spot-on applications. Both, a single application four weeks before inoculation and four applications at monthly intervals before inoculation, were highly efficacious. The results demonstrate that the persistent efficacy of moxidectin against L3/L4 of *A. vasorum* over a four week period can already be achieved with a single application of Advocate® spot-on. This is due to the special pharmacokinetic profile of moxidectin. After application of Advocate® spot-on, serum moxidectin reaches a Cmax about four to nine days post application. Subsequently the serum level declines but is still significant at day 28 post application (Bowman et al. 2016). Comparing these data with the data of another macrocyclic lactone, milbemycin oxime, Holstrom et al. (2011) found a much faster decline in the plasma concentration of milbemycin's major active component milbemycin A4 5-oxime. After oral administration Tmax ranged between three and six hours and the half-life was approximately 57 hours. This may also explain why, even under regular monthly treatment with the licensed doses of milbemycin - used here in a combination product containing milbemycin and afoxolaner (NexGard Spectra®, Merial), - infection with adult worms could not be completely prevented if experimental infections with L3 of *A. vasorum* were performed at weekly intervals. Nine out of 10 dogs still harboured 1–24 worms, although an overall efficacy of 94.9 % was observed in the study (Lebon et al. 2016).

For *A. vasorum* the elimination of larval stages is of importance not only to prevent the development of mature nematodes and thus a patent infection but also to prevent the damage of lung tissue already caused by the immature adults within the first four weeks after infection.

In our study, it could be demonstrated that the efficacy of Advocate® against L3/L4 larvae resulted in macroscopic unremarkable lungs at necropsy 70–72 days after inoculation, whereas seven of

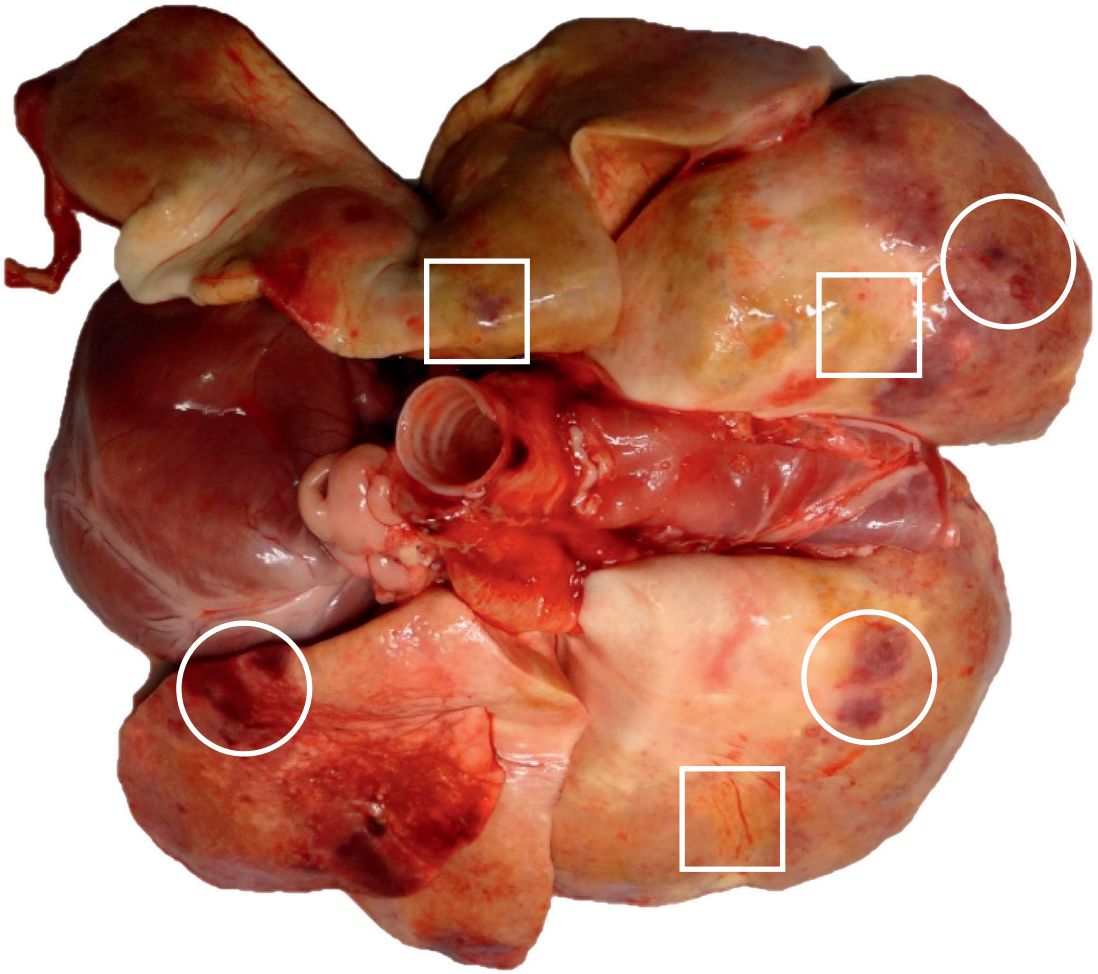

Fig. 2 Pathological lesions of a lung from a dog of the untreated control group inoculated with 250 third stage-larvae of *Angiostrongylus vasorum* and necropsied on day 72.
Lung shows lobar verminous pneumonia with spotty hyperemia (circles) and broad atelectasis in reddish margins of cranial and caudal lobes. Subpleural yellowish areas (squares) indicate haemosiderin deposition.

eight dogs in the untreated control group showed lung tissue pathology typical of angiostrongylosis (Fig. 2).

The importance of killing of the early lungworm stages (L3/L4) is also elucidated by the results of the study performed by Schnyder et al. (2009), who treated eight dogs each at four and 32 days post infection respectively with Advocate® spot-on. None of the treated dogs developed a patent infection, with no worms being detected in any of the dogs at necropsy on days 56–59. Advocate® was shown to be 100 % effective against L4 and immature adults of *A. vasorum*. The study also demonstrated that an early treatment at day four post infection (to kill L4 larvae) prevented the development of tissue damage in the lung of the dogs whereas in the group where development to the immature adult stage was allowed to take place, disseminated remnants of granulomatous inflammation could be detected by histological examination of lung tissue in seven of eight of the dogs which went on to be successfully

treated at 32 days post infection. In four of these eight dogs also arterial thrombi could be found. Growing prevalence in dogs and wildlife has been reported for *A. vasorum* (Taylor et al. 2015, Barutzki et al. 2017). There is also evidence for a growing infection rate in snails (Aziz et al. 2016). Nowadays, improved diagnostic techniques are available which are less labour- intensive and more sensitive than the classical Baermann migration-sedimentation technique in detecting L1 of *A. vasorum* in faeces (Schnyder et al. 2011). The spread of the infection and improved diagnostics have led to a growing awareness of *A. vasorum* infection in dogs. *A. vasorum* infections cause severe respiratory distress, coagulopathies and a wide range of other clinical signs in dogs; they may even cause death. Even immature adults of the nematode cause lung tissue damage at a time when clear clinical symptoms may still be absent and diagnostic techniques cannot yet provide reliable results. Complete prevention of infection with the worm is therefore desirable. The present study demonstrates that Advocate® completely prevents establishment of the *A. vasorum* parasite, ensuring dogs remain free of all immature and adult stages. In addition, Advocate® kills the parasite before lung damage can occur and patency is completely prevented, ensuring that there is no shedding of L1 larvae. In endemic areas, regular monthly treatment throughout the period of the dog's potential exposure to infected snails and slugs will protect the dog from infection.

Ethical standards

The study was performed in compliance with current national laws and regulations.

Funding

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Conflict of interest

Claudia Böhm, Gabriele Petry, Franziska Barthel and Roland Schaper are employees of Bayer Animal Health GmbH. Holger Schmidt is the owner of BioMedVet Research GmbH. Katharina Raue is employee of the Institute for Parasitology, University of Veterinary Medicine Hannover, Germany.

References

Aziz NAA, Daly E, Allen S, Rowson B, Greig C, Forman D (2016) Distribution of *Angiostrongylus vasorum* and its gastropod intermediate hosts along a rural-urban gradient in two cities in the United Kingdom, using real time PCA. Parasit Vectors 9: 56

Barutzki D, Dyachenko V, Schaper R (2017) Lungworms in Germany 2002–2016 – Is there an increase in occurrence and geographical spread? Parasitol Res 116: S11–S30

Bolt G, Monrad J, Koch J, Jensen AI (1994) Canine angiostrongylosis: a review. Vet Rec 107: 447–452

Bowman DD, Grazette AR, Basel C, Wang Y, Hostetler JA (2016) Protection of dogs against canine heartworm infection 28 days after four monthly treatments with Advantage Multi® for Dogs. Parasit Vectors 9: 12–18

Bowman DD, Ohmes CM, Hostetler JA, Keil DJ, Settje TL,Charles SD (2017) Efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) for the prevention of heartworm disease and infection all month long. Parasit Vectors Supplement. In press

Conboy G, Guselle N, Schaper R (2017) Spontaneous shedding of metastrongyloid third-stage larvae by experimentally infected *Limax maximus*. Parasitol Res 116: S41–S54

Elsheika HM, Holmes SA, Wright I, Morgan ER, Lacher DW (2014) Recent advances in the epidemiology, clinical and diagnostic features, and control of canine cardio-pulmonary angiostrongylosis. Vet Res 45: 92–103

European Medicines Agency a: Summary of Product Characteristics Advocate, last updated 03/03/2017, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000076/WC500060917.pdf accessed 02.04.2017 a

European Medicines Agency b: EPAR Scientific discussion Advocate, published 27/07/2009, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/veterinary/000076/WC500060915.pdf, accessed 02.04.2017 b

Ferdushy T, Tabarak H (2010) *Angiostrongylus vasorum*; the "French Heartworm". Parasitol Res 107: 765–773

Genchi C, Genchi M, Petry G, Kruedewagen EM, Schaper R (2013) Evaluation of the efficacy of Imidacloprid 10 % / Moxidectin 2.5 % (Advantage® Multi, Bayer) for prevention of *Dirofilaria repens* infection in dogs. Parasit Res 112: 81–89

Holstrom SD, Totten ML, Newhall KB, Qiao M, Riggs KL (2011) Pharmacokinetics of spinosad and milbemycin oxime administered in combination and separately per os to dogs. J vet Pharmacol Therap 35, 351–364

Hurníková Z, Miterpáková M, Mandelík R (2013) First autochthonous case of canine *Angiostrongylus vasorum* in Slovakia. Parasitol Res 112: 3505–3508

Jacobs DE, Arakawa A, Courtney CH, Gemmell MA, McCall JW, Myers GH, Vanparijs O (1994) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of anthelmintics for dogs and cats. Vet Parasitol 52: 179–202

Lebon W, Tielemans E, Rehbein S, Dumont P, Yoon S, Beugnet F, Jeannin P, Larsen D, Halos L (2016) Monthly administrations of milbemycin oxime plus afoxolaner chewable tablets to prevent *Angiostrongylus vasorum* infection in dogs. Parasit Vectors 9: 485

Lempereur L, Martinelle L, Bayrou C, Marechal F, Bayrou C, Dalemans AC, Schnyder M, Losson BJ (2016) Prevalence of *Angiostrongylus vasorum* in Southern Belgium, a coprological and serological survey. Parasit Vectors 9: 533

Penagos F, Gutiérrez J, Velez JD, Piedrahita D, Lange M, Hermosilla C, Taubert A, Chaparro J (2017) First report of *Angiostrongylus vasorum* infections in dogs as well as in the neozoan intermediate host (*Achatina fulica*) in Medellín, Colombia. Parasit Vectors 10 (Suppl 1): 5 A 46

Schnyder M, Fahrion A, Ossent P, Kohler L, Webster P, Heine J, Deplazes P (2009) Larvicidal effect of imidacloprid/moxidectin spot-on solution in dogs experimentally infected with *Angiostrongylus vasorum*. Vet Parasit 166: 326–332

Schnyder M, Tanner I, Webster P, Barutzki D, Deplazes P (2011) An ELISA for sensitive and specific detection of circulating antigen of *Angiostrongylus vasorum* in serum samples of naturally and experimentally infected dogs. Vet Parasit 179: 152–158

Taylor CS, Gato RG, Learmount J, Aziz NA, Montgomery C, Rose H, Coulthwaite CL, McGarry JW, Forman DW, Allen S, Wall R, Morgan ER (2015) Increased prevalence and geographic spread of the cardiopulmonary nematode *Angiostrongylus vasorum* in fox populations in Great Britain. Parasitology 142: 1190–1195

Traversa D, di Cesare A, Meloni S, di Regalbono AF, Milillo P, Pampurini F, Venco L (2013) Canine angiostrongylosis in Italy: occurrence of *Angiostrongylus vasorum* in dogs with compatible clinical pictures. Parasitol Res 112: 2473–2480

Willesen JL, Kristensen AT, Jensen AL, Heine J, Koch J (2007) Efficacy and safety of imidacloprid 10 % and moxidectin 2.5. % spot-on solution and fenbendazole in the treatment of dogs naturally infected with *Angiostrongylus vasorum* (Baillet, 1866). Vet Parasitol 147: 258–264