



Acute death in heartworm-infected cats: Unraveling the puzzle

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Abstract

Although the acute death syndrome in feline heartworm disease is widely recognized, its pathogenesis remains a mystery. The most widely held hypothesis is that an acute anaphylactic reaction, perhaps precipitated by the death of the parasite, is the underlying cause. This study investigated the role of the physical form of antigen (Ag) in the ensuing reaction when *Dirofilaria immitis*-sensitized cats are challenged by intravenous (IV) administration of heartworm Ag. Healthy *D. immitis*-naïve cats ($n = 23$) were sensitized using subcutaneous injections of adjuvanted *D. immitis* Ag administered weekly for 6 weeks. After sensitization, cats ($n = 20$) were anaesthetized and challenged with IV *D. immitis* Ag in various forms or with IV sterile 0.9% saline ($n = 3$). Systolic blood pressure, respiratory rate, degree of dyspnea, blood oxygen saturation, and heart rate were measured immediately before and at 10–15 min intervals after challenge until terminal apnea occurred or until euthanasia at 140 min after challenge. Blood samples were collected for complete blood count and measurements of serum serotonin immediately before and at 10, 20, and 35 min after challenge. Clinical observations were recorded as they occurred, or at 10–15 minute intervals, whichever was the more frequent. The most severe post-challenge reactions occurred in cats challenged with Ag from dead worms, live worms, and 20 ng/mL Ag. Dyspnea increased significantly after challenge in all three groups ($p < 0.001$; $p = 0.04$, and $p = 0.002$, respectively), and blood oxygen saturation dropped post-challenge in the Dead Worm ($p < 0.001$) and the 20 ng/mL Ag ($p = 0.002$) groups. In the 20 ng/mL Ag group, systolic blood pressure decreased ($p < 0.05$) and respiratory rate increased ($p < 0.05$) post-challenge. Clinical observations included dyspnea, gastrointestinal signs (retching, defecation, or flatulence), urination, and less commonly, hemorrhage from the nostrils or anus, or cutaneous swelling (general or specifically facial). The 20 ng/mL Ag group had the highest rate of clinical signs, followed by the Dead Worm group. The most common and reliable hematologic change associated with severe clinical effects of *D. immitis* Ag challenge was increased hematocrit, which was statistically higher after challenge than at baseline in the Dead Worm group ($p = 0.012$). The model demonstrated that the physical form of heartworm Ag used for IV challenge in *D. immitis*-sensitized cats is an important factor for determining the characteristics of the post-challenge reaction, and the amount of exposed internal filarial Ag presented to the feline immune system may influence the severity of the response to challenge.

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1. Introduction

Acute death has been widely reported in asymptomatic heartworm-infected cats (Dillon, 1984; Holmes, 1993; Dillon, 1995; Ralston et al., 1998; Court et al., 2000), but there is minimal information concerning the pathogenesis involved in these cases. Acute collapse

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may occur with or without previous clinical signs and with infections by as few as one worm (Dillon, 1998b). Acute collapse is a much more common clinical presentation in feline heartworm disease than in canine heartworm disease (Ralston et al., 1998). In one Australian report, 21 of 45 cases of feline heartworm disease (47%) exhibited acute death either at home or on arrival at the veterinary clinic (Court et al., 2000). Dillon (personal communication, 1999) estimates that the corresponding figure for the United States is approximately 30%. These figures may overestimate the prevalence of heartworm-induced acute death syndrome since acute death is more readily diagnosed and recalled than feline heartworm infection.

Acute death in cats infected with *Dirofilaria immitis* has been attributed to circulatory collapse and respiratory failure from acute pulmonary arterial infarction (Dillon, 1984), specifically acute pulmonary thromboembolism as a result of spontaneous death of adult heartworms (Dvorak, 2000). However, in the acute death syndrome, filariae are not always found embolizing the main pulmonary arteries (Dillon, 1998a), and radioisotope studies have demonstrated that lung lobes are rarely ischemic (Dillon, 1999). It has been suggested that pulmonary hypertension may also play a role, but it is rarely present in affected cats, at least based on the presence of secondary signs, such as right-sided heart failure and congestive heart failure (Lok and Knight, 1998).

Surgical extraction procedures complicated by the accidental dissection of filariae have resulted in an acute shock-like reaction and death of the cat. It is hypothesized that the damage to the heartworm cuticle causes the sudden release of large amounts of heartworm Ag, resulting in acute systemic anaphylaxis. Brown et al. (1999) provided a report of a cat that responded to corticosteroid and atropine injections given in an attempt to treat suspected resultant anaphylaxis.

The purpose of this study was to evaluate reactions of *D. immitis*-sensitized cats challenged IV with *D. immitis* Ag and to determine whether the severity of their reactions is related to the physical form and amount of Ag administered.

2. Materials and methods

2.1. *D. immitis* sensitization and challenge procedures

Approval for this study was granted by The University of Queensland Animal Ethics Committee (AEC No.

SVS/053/URG/BEQ/SIMVEX/PHD). Healthy *D. immitis*-naïve cats ($n = 23$) were selected from a larger group of 42 and were sensitized using subcutaneous injections of adjuvanted heartworm antigen (Ag) administered once weekly for 6 weeks. After sensitization, cats ($n = 20$) were anaesthetized and challenged with intravenous (IV) *D. immitis* Ag presented in various physical forms (Table 1). A control group ($n = 3$) was sham-challenged with 20 mL of sterile 0.9% saline by IV injection. Further details of the sensitization protocol and experimental methods have been previously published (Litster and Atwell, 2006).

2.2. Serum anti-*D. immitis* IgE concentration

Whole blood samples were collected from five randomly selected cats from the study group ($n = 23$), and serum was harvested from each sample immediately prior to the first Ag inoculation and then weekly for 5 weeks for determination of serum anti-*D. immitis* IgE concentrations. Anti-*D. immitis* IgE concentrations also were determined for seven additional cats immediately prior to challenge. Serology was performed at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC. Serum *D. immitis*-specific assays were performed using a modification of a previously published technique (Hammerberg et al., 1997). Microtiter styrene “U” bottom 96-well plates were coated overnight at 4 °C with 50 µL/well of adult *D. immitis* extract at 50 µg/mL in carbonate/bicarbonate buffer (0.1 M pH 9.6). Plates were washed three times with phosphate-buffered saline (0.05 M PO₄, pH 7.4) plus 0.1% Tween® 20 (PBS-0.1T), using 2-min incubations between washings. The wells were each blocked with 200 µL of 1% (w/v) nonfat dry milk in PBS-0.1T either at room temperature for 2 h or overnight at 4 °C. Plates were washed once

Table 1
Experimental design

Name of group	Number of cats	Method of challenge
Control	3	IV injection of normal saline
Dead worm	7	IV insertion of preserved dead transected filariae
Live worm	6	IV insertion of whole live filariae
Incubation fluid	3	IV injection of fluid of unknown Ag concentration from whole untransected filariae
20 ng/mL Ag	4	IV injection of fluid of known Ag concentration from transected filariae to result in a plasma Ag concentration of 20 ng/mL

with PBS-0.1T. Cat sera diluted 1:5 with PBS-0.1T were applied in triplicate at 50 μ L/well. Plates were incubated at room temperature for 2 h and then washed five times with PBS-0.1T. Biotinylated mouse mAb 5.91 in PBS-0.1T containing 10 μ g/mL dog IgG and 10% mouse serum at 4 μ g/mL were added to the plate at 50 μ L/well and incubated for 2 h at room temperature. The addition of dog IgG reduces non-specific binding by mAb 5.91. Plates were washed five times with PBS-0.1T before avidin peroxidase diluted 1:2000 in PBS-0.1T containing 1% (w/v) bovine serum albumin was added (50 μ L/well) and incubated for 30 min. Plates were washed five times with PBS-0.1T before ABTS substrate was added (50 μ L/well). Absorbance at 405 nm was read at 60 min and at 16 h. Canine IgE was used to generate a linear standard curve where optical density (OD) related directly to the weight of IgE trapped. Standard regression analysis was used to calculate relative absorbance units of IgE from OD, which were multiplied by five to account for the 1:5 dilution.

2.3. Serum anti-*D. immitis* IgG concentrations

Serum anti-*D. immitis* IgG titers were performed in 12 cats immediately prior to the first Ag inoculation and were repeated weekly for five weeks. Sera from 5 of these 12 cats were also assayed for anti-*D. immitis* IgE titers at the same times (see 2.2). Serum anti-*D. immitis* IgG titers were determined for 42 study cats (including the 23 that were selected to participate in the study) immediately prior to challenge. Because *D. immitis* is exotic to New Zealand, stored sera from eight cats from a veterinary practice in New Zealand were also tested for anti-*D. immitis* IgG titers. These eight sera samples acted as negative controls for the pre-inoculation sera collected from the experimental cats. *D. immitis* antigen (AGEN Biomedical Ltd.) was diluted in PBS to a final concentration of 10 μ g/mL, and 50 μ L of feline serum was added to each well of a 96-well ELISA plate. The plate was tapped gently and agitated, covered with wax film, and incubated at room temperature for 1 h. The contents of the plate were then emptied and the wells flooded with PBS + 0.5 g/L Tween (PBS-0.5T), incubated at room temperature for 2 min, and emptied. This last step was repeated twice before the plate was struck against paper towels to empty the contents of the wells.

One hundred microliters of a 1:100 dilution of feline serum in PBS-0.1T was added to Column 1, rows A–H inclusive. Fifty microliters of PBS-0.5T was added to all remaining wells. Fifty microliters of serum was then transferred from Column 1 to Column 2. This step was

repeated across the plate through Column 11 so that each column contained exactly half as much serum as the previous one (i.e. doubling dilutions). Column 12 acted as a control column to detect any conjugation between the *D. immitis* antigen coating layer and the anti-cat HRP. The plate was tapped gently and agitated, covered with wax film, and incubated at room temperature for 15 min. The contents of the plate were then emptied and the wells were flooded with PBS-0.5T, incubated at room temperature for 2 min, and emptied. This last step was repeated twice before the plate was struck against paper towels as above.

Anti-cat horseradish peroxidase (HRP) was diluted 1:1000 in PBS-0.5T, and 50 μ L/well of this solution was added to Columns 1 through 12. The plate was tapped gently and agitated, covered with wax film, and incubated at room temperature for 15 min. The contents of the plate were then emptied and the wells were flooded with PBS-0.5T, incubated at room temperature for 2 min, and emptied. This last step was repeated twice before the plate was struck against paper towels as above.

ABTS was activated by adding 10 μ L of 3% hydrogen peroxide per 10 mL of ABTS just before the activated ABTS was required. One hundred microliters of activated ABTS was added to all 96 wells and incubated at room temperature for 10–20 min until optimum colorization of the wells was achieved. The reaction was stopped by adding 50 μ L of 3.9% oxalic acid to all wells, and the plate was read at 420 nm (Lab Systems, Helsinki, Finland). A printout was generated detailing absorbance units for each of the serial dilutions. The anti-*D. immitis* IgG titer of each sample was read as the highest dilution giving a value of greater than 0.1 absorbance units.

2.4. Heartworm antigen preparation

To obtain heartworm Ag, adult *D. immitis* were collected at necropsy of infected dogs surrendered for euthanasia. To prepare filariae for the group challenged with dead worms (Dead Worm group), each filaria was broken into two segments using a scalpel blade to transect it approximately halfway along its length. Filarial viscera emanated from the site of transection. The filarial segments were then individually suspended in 20 mL sterile saline in sealed zip lock plastic bags. The bags were then stored at -20°C until 1 h before use, when the bag was thawed at room temperature. On the day of challenge, the thawed filariae were visually assessed for damage before being aspirated in sterile saline into a 12 G $\times$ 9-cm cannula (Dwellcath; Tuta

Laboratories) connected to a 20-mL syringe containing 20 mL sterile saline.

To prepare filariae for the group challenged with live worms (Live Worm group), adult *D. immitis* were collected at necropsy as described previously and carefully washed in sterile PBS, before being placed in 50–100 mL of sterile heartworm culture medium in individual sterile screw top containers. This medium consisted of 100 mL RPMI 1640 with 1 mL of stock solution of penicillin/streptomycin (containing 10,000 IU penicillin/mL and 10,000 µg streptomycin/mL) to give a final concentration of 100 IU penicillin and 100 µg streptomycin/mL. The containers were then placed in an incubator at 37 °C in 5% carbon dioxide with lids loosely placed over them. Each filaria was then assessed for independent movement. On the day of challenge, a 2-mm (6 F) × 50-cm dog catheter connected to a 20-mL syringe containing 20 mL sterile saline was used to aspirate a filaria into the catheter. Care was taken to ensure that the filaria remained threaded inside the shaft of the catheter.

To prepare fluid for the group to be challenged with incubation fluid (Incubation Fluid group), adult *D. immitis* were collected at necropsy as described earlier and washed carefully with sterile saline. Entire live filariae were placed in individual containers of 25 mL sterile saline and left at room temperature for 48 h. The filariae exhibited no further independent movement from approximately 2 h after they were placed in the saline. Aliquots of incubation fluid were then collected from each container hourly during the following 24 h and analyzed quantitatively for *D. immitis* antigen concentration (AGEN Biomedical). Filariae were carefully removed from the fluid after 48 h. The containers of incubation fluid were then stored frozen before being thawed at room temperature on the day of challenge. An additional six specimen jars, each containing 25 mL sterile saline were analyzed quantitatively for *D. immitis* antigen concentration at hourly intervals as described for the incubation fluid and acted as negative controls.

To prepare fluid for the 20 ng/mL Ag group, adult *D. immitis* were collected at necropsy as described above. Individual filariae were placed in separate sterile containers of 25 mL saline. After one hour at room temperature, each filaria was transected using a scalpel blade. Transected filaria then remained in the containers for a further 23 h before they were removed. Aliquots of fluid were then collected from each container hourly for the following 24 h and were analyzed quantitatively for *D. immitis* antigen concentration before being stored frozen as described for the incubation fluid. On the day

of challenge, the fluid was thawed at room temperature. At challenge, a volume of fluid calculated to result in a plasma concentration of 20 ng *D. immitis* antigen/mL was injected IV via the jugular cannula in each cat. This calculation was based on the following equation

$$\text{Plasma volume (mL)} = 38.5 \times \text{bodyweight (kg)}.$$

2.5. Pre- and post-challenge physiologic and hematologic measurements and clinical observations

Systolic blood pressure (measured indirectly using Doppler), respiratory rate, degree of dyspnea, blood oxygen saturation and heart rate were measured immediately before and at 10–15-min intervals after challenge until terminal apnea occurred or until euthanasia at 140 min after challenge. Blood was collected for complete blood count immediately before and at 10, 20, and 35 min after challenge. Further details of physiologic and hematologic measurement methods have been previously published (Litster and Atwell, 2006). Clinical observations were recorded as they occurred, or every 10–15 min, whichever was the more frequent.

2.6. Serum serotonin

A blood sample was collected from each cat for serum serotonin assay immediately before and at 10, 20, and 35 min after challenge. Serum serotonin assays were performed by allowing the blood sample to clot, which causes the platelet cell membranes to rupture and release stored cytoplasmic serotonin into the serum. The serum was then assayed for serotonin. Using this technique, serum serotonin levels are high when platelets have high levels of stored cytoplasmic serotonin and low when platelets have already released stored cytoplasmic serotonin in response to a previous triggering event, since the half-life of serotonin in feline serum is 1.03 min (Siepler et al., 1980).

2.7. Statistics

Statistical analysis was performed using computer software (Sigmastat Statistical Software Version 2.0; Jandel Corporation). Baseline and post-challenge data for each physiologic measurement were compared within each study group, and post-challenge measurements for each group were compared with those for the control group. Serum serotonin was compared between baseline and 10 min after challenge. Comparisons were

made using Student's *t*-tests if normality and equal variance tests passed. Mann–Whitney rank sum tests were used if normality or equal variance tests failed or if data were non-parametric. Data are represented graphically.

3. Results

3.1. Serum anti-*D. immitis* IgE concentration

The mean anti-*D. immitis* IgE titer measured for cats immediately before challenge was significantly higher than their pre-inoculation mean ($p = 0.016$) (Fig. 1).

3.2. Serum anti-*D. immitis* IgG concentration

Fig. 1 shows the *D. immitis*-specific IgG response to the course of Ag inoculation. Mean serum anti-*D. immitis* IgG titers immediately before challenge were significantly higher than those measured pre-inoculation ($p < 0.001$) but were lower than titers for sera collected on the last day of the course of Ag inoculation ($p = 0.018$). The interval between the last day of the course of inoculation and challenge was staggered by 3–8 weeks for individual cats. Sera from the eight cats used as negative controls (dilution 1:10) contained an anti-*D. immitis* IgG titer of 640.00 ± 104.75 (mean $\pm$ S.E.). The positive anti-*D. immitis* IgG titers in the cats from the New Zealand practice were not related to anti-cat IgG HRP binding on the ELISA plate, since the controls containing no serum gave negative results. Antibody titers for the New Zealand cats were significantly higher than the pre-inoculation titers for the experimental cats ($p = 0.012$).

3.3. In vitro *D. immitis* Ag concentrations

No *D. immitis* Ag was detected in the incubation fluid prepared for the Incubation Fluid group or in the

six jars of sterile saline at any time throughout the 24-h measurement period. In fluid prepared for the 20 ng/mL Ag group, the *D. immitis* Ag concentration rose sharply from 0.0 ± 0.0 ng/mL (mean $\pm$ S.E.) before transection to 489.1 ± 42.2 ng/mL immediately after transection and remained at a similar concentration for the remainder of the study. All filariae ceased independent movement within the first hour of incubation.

3.4. Pre- and post-challenge physiologic and hematologic measurements

Changes in physiologic parameters and hematocrit pre- and post-challenge by study group are shown in Fig. 2. Systolic blood pressure was significantly lower post-challenge in all groups compared with that for the control group (Dead Worm challenge: $p < 0.001$; Live Worm challenge: $p < 0.001$; Incubation Fluid challenge: $p = 0.027$; 20 ng/mL Ag challenge: $p < 0.001$). Post-challenge respiratory rate in the Dead Worm group ($p = 0.006$) and the 20 ng/mL Ag group ($p < 0.001$) were significantly higher than those for the control group.

In the 20 ng/mL Ag group, post-challenge systolic blood pressure was significantly lower ($p = 0.013$) and respiratory rate was significantly higher ($p = 0.043$) compared with baseline values. Post-challenge degree of dyspnea was higher (Dead Worm group: $p < 0.001$; Live Worm group: $p = 0.004$; 20 ng/mL Ag group: $p < 0.001$) and blood oxygen saturation was lower in all groups (Dead Worm group: $p < 0.001$; Live Worm group: $p = 0.004$; 20 ng/mL Ag group: $p < 0.001$), except the Incubation Fluid group, when compared with values for the control group.

There were significant post-challenge increases in dyspnea within the Dead Worm group ($p < 0.001$), the Live Worm group ($p = 0.043$) and 20 ng/mL Ag group ($p = 0.002$) and reductions in blood oxygen saturation in the Dead Worm ($p < 0.001$) and 20 ng/mL Ag groups ($p = 0.002$) (Fig. 2).

Heart rate post-challenge was lower in the Dead Worm ($p < 0.001$) and Incubation Fluid ($p = 0.006$) groups than in the control group (Fig. 2). There was a significant reduction in heart rate after challenge within the Live Worm ($p = 0.014$) and Incubation Fluid ($p = 0.009$) groups. The post-challenge hematocrit mean was significantly higher in the Dead Worm ($p < 0.001$) and 20 ng/mL Ag ($p = 0.001$) groups than in the control group, and hematocrit increased significantly within the Dead Worm group ($p = 0.012$) after challenge.

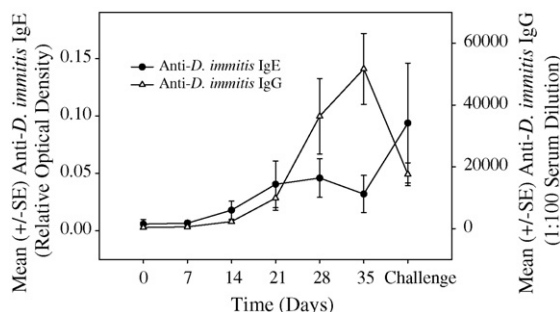


Fig. 1. The plot with solid dots should be for anti-*D. immitis* IgE and the plot with open triangles should be for anti-*D. immitis* IgG.

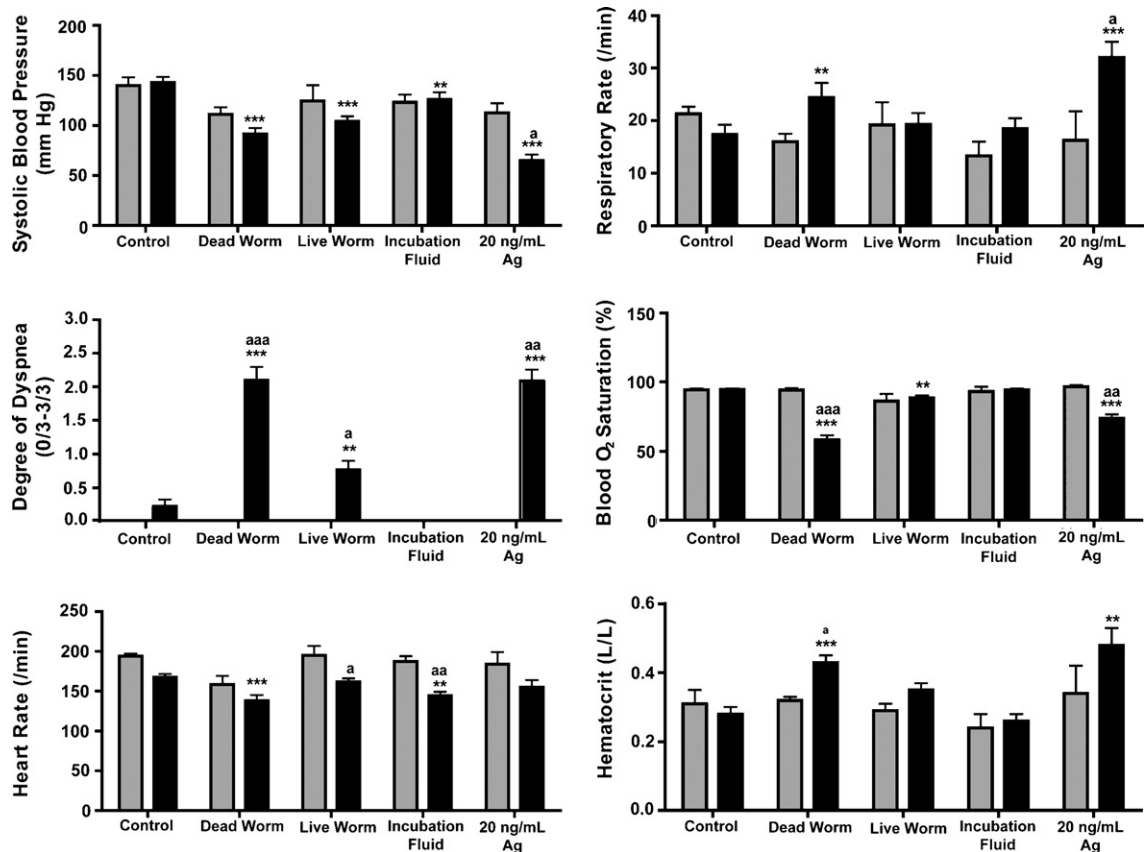


Fig. 2. Baseline (gray bars) and post-challenge (black bars) physiologic and hematologic measurements and clinical observations in sensitized cats challenged with *Dirofilaria immitis*. Pre-challenge vs post-challenge by group: a = $p < 0.05$; aa = $p < 0.01$; aaa = $p < 0.001$. Post-challenge comparison of challenge groups with control group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. All baseline measurements for degree of dyspnea were 0.00 ± 0.00 . Data are shown as mean $\pm$ S.E.

3.5. Pre- and post-challenge clinical observations

Clinical observations included dyspnea; gastrointestinal signs, such as retching, defecation, or flatulence; urination; hemorrhage from the nostrils or anus; and cutaneous swelling (general or specifically facial). Dyspnea and gastrointestinal signs were the most frequent clinical observations. These clinical events

occurred only after challenge. The frequency of these clinical signs post-challenge is presented in Table 2. The 20 ng/mL Ag group had the most cats showing clinical signs, followed by the Dead Worm group. In the Live Worm group, four of the five cats showed sustained dyspnea, and one cat showed gastrointestinal signs. There were no clinical observations in the Incubation Fluid group or the Control group.

Table 2

Frequency of post-challenge clinical observations in groups of cats challenged with *Dirofilaria immitis* antigen

Challenge group	Sustained dyspnea ^a	Retching	Defecation/flatulence	Urination	Hemorrhage from nostrils/anus	Cutaneous swelling
Control	4/7	1/7	2/7	1/7	0/7	0/7
Dead worm	4/5	0/5	1/5	0/5	0/5	0/5
Live worm	0/3	0/3	0/3	0/3	0/3	0/3
Incubation fluid	4/4	4/4	3/4	1/4	1/4	0/4
20 ng/mL Ag	0/3	0/3	0/3	0/3	0/3	0/3

Data are shown as number with observations over the total number of cats challenged.

^a Dyspnea present for at least two consecutive measurement times.

3.6. Serum serotonin

Using the current assay method, serum serotonin levels are high when platelets have high levels of stored cytoplasmic serotonin and low when platelets have already released stored cytoplasmic serotonin in response to a previous triggering event. There was a significant reduction in serum serotonin in the Dead Worm ($p = 0.03$) and 20 ng/mL Ag ($p = 0.017$) groups after challenge, suggesting the previous release of stored platelet serotonin in these groups. Serum serotonin values were not significantly different after challenge in the control, Incubation Fluid, or Live Worm challenge groups ($p > 0.05$).

4. Discussion

This study has demonstrated that in this experimental model of the acute death syndrome in heartworm-sensitized cats, the physical form of *D. immitis* is an important factor that determines the characteristics of the ensuing reaction. Increased degree of dyspnea and reduced blood oxygen saturation and blood pressure were the primary effects of *D. immitis* Ag challenge, and the magnitude of these changes reflected the clinical severity and speed of the post-challenge reaction. Interestingly, heart rate was significantly reduced in the control and Live Worm challenge groups, which were the two groups that showed the fewest alterations in other physiologic parameters during the study procedure. It is possible that the effects of prolonged deep general anesthesia in the cats in these two groups (which was longer than for cats in other groups because their survival time was longer) may have been responsible for reduced heart rate, whereas increased sympathetic tone and reduced time to the study endpoint in these more severely affected groups may have reduced this cardiac effect. The most reliable hematologic change reported after Ag challenge was increased hematocrit. Either or both of two hypotheses could explain this. Fluid shift out of the intravascular space during anaphylaxis could result in hemoconcentration (Noli and Miolo, 2001). Alternatively, increased sympathetic tone due to hypoxia during acute shock could result in splenic contraction and the release of stored erythrocytes into the circulation (Blue and Weiss, 1981).

Clinical observations after Ag challenge are consistent with those of feline acute systemic anaphylaxis reported in the literature (McCusker and Aitken, 1966; Jankovic et al., 1969; Szilagyi et al., 1973; Plunkett, 2000; Litster and Atwell, 2006). The number of clinical

observations recorded for each group reflected the severity of other physiologic and hematologic changes.

When the physical form of the inciting Ag was considered, the most severely affected groups were the 20 ng/mL Ag group and the Dead Worm group. The group challenged with live worms was relatively mildly affected and that challenged with incubation fluid showed no discernable effects of Ag challenge. We hypothesize that the exposure of internal filarial antigens could have triggered a severe reaction when transected filariae or their fluid was administered IV to previously sensitized cats. Accordingly, exposure to whole live untraumatized filariae or fluid in which such filariae were incubated was not associated with the release of internal filarial antigens; therefore, sudden and dramatic derangements in physiologic, hematologic, and clinical parameters did not occur. In vitro *D. immitis* Ag tests performed in this study confirmed that trauma to the filarial parasites results in substantial release of Ag, in contrast to atraumatic handling, which was not associated with any Ag release. Recent published studies have implicated the bacterial endosymbiont *Wolbachia*, present in all *D. immitis* filariae, in the development of the pathology and in the regulation of the host's immune response to heartworm infection (Simón et al., 2007). While this relationship has been investigated in the dog (Kramer et al., 2005a,b), its role in feline heartworm infection is yet to be fully explored and may have relevance to the experimental model reported here.

Serum serotonin followed other parameters in that it changed significantly in the two most severely affected groups. It has been demonstrated in both in vivo and in vitro studies that serotonin is an important mediator of acute airway smooth muscle contraction in type-1 hypersensitivity reactions in cats (Padrid et al., 1995a,b). Serotonin is also known to incite the Bezold–Jarisch reflex (Aviado and Guevara Aviado, 2001), a combination of profound hypotension, bradycardia, and apnea. These clinical effects are identical to those observed in the more severely affected cats in this experimental model and it is hypothesized that the Bezold–Jarisch reflex may play a role in the pathogenesis of the clinical response in these cats. Whether either or both of these pathophysiologic mechanisms are responsible for the clinical signs of acute shock in cats, it is possible that serotonin-blocking drugs could play a useful role in the prevention of the acute shock-like reaction that occurs when filariae are accidentally dissected during surgical extraction procedures. Further manipulation of the experimental model reported here with an anti-serotonin drug such as

cypothetadine could be a useful and interesting exploration of this concept.

While the course of inoculations used in this model produced a statistically verifiable anti-*D. immitis* IgE and anti-*D. immitis* IgG response, the role of this response in the ensuing reaction after challenge with heartworm Ag has yet to be confirmed. This is the main limitation of this study and could be rectified by the addition of a group of unsensitized cats, challenged with IV *D. immitis* Ag as described here.

In conclusion, this model has demonstrated that sensitized cats challenged IV with heartworm Ag undergo an acute shock-like reaction, and that the severity of the reaction is related to the physical form and amount of *D. immitis* Ag administered.

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Conflict of interest

Authors Litster, Atwell, and Atkins report no external funding, so there are no conflicts of interest.

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