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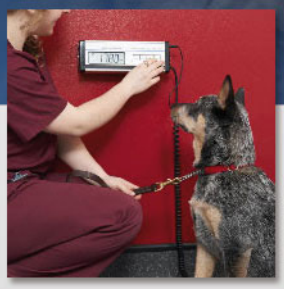

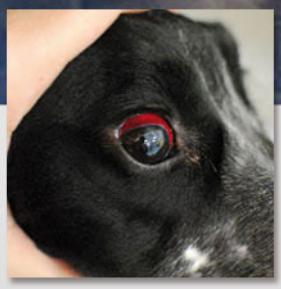

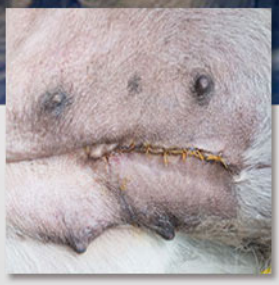

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Each scientific article is classified with one or more icons.

These refer to the species (in green) of animal or the veterinary discipline (in blue) relevant for the article.


Dogs


Cats


Dogs and Cats/
Small animals


Rabbits


Less common pets


Anaesthesia


Bacterial Diseases


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Dermatology


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Ear Nose Throat


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Zoonosis

Reprint paper*

Innocent Cardiac Murmur in Puppies: Prevalence, Correlation with Haematocrit and Auscultation Characteristics

Viktor Szatmári[1], Martin W. van Leeuwen and Erik Teske

SUMMARY

Background: The aims of this study were to establish the prevalence of innocent cardiac murmurs in clinically healthy puppies, to investigate a possible correlation between the presence of an innocent murmur and haematocrit, and to describe the auscultation characteristics of innocent murmurs.

Hypothesis: Lower haematocrit contributes to the genesis of innocent murmurs.

Animals: Five hundred and eighty-four client-owned clinically healthy puppies, between 20 and 108 days old.

Methods: Two cross-sectional surveys with a 1-year (n = 389 pups) pilot and a half-year (n = 195 pups) principal study periods. Cardiac auscultation was performed by a single, board-certified cardiologist. Haematocrit was measured with an automatized haematology analyser. Echocardiography was performed only on puppies with a cardiac murmur in the principal study.

Results: In the pilot study, 15% of the dogs had a murmur. Innocent murmur was diagnosed in 28% of the 195 dogs in the principal study. Innocent murmurs were systolic, mostly with a musical character and with a maximal intensity of 2 of 6, and mostly with the point of maximal intensity in the left cardiac base. The haematocrit was significantly lower in the group with a murmur compared to the group without (P = 0.023).

Conclusions and Clinical Importance: Innocent murmur was a common finding in puppies at the age when the first veterinary controls usually take place. Physiologic anaemia contributes to the genesis of innocent murmurs in puppies. Rising haematocrit in growing puppies can explain the spontaneous disappearance of innocent murmurs with aging. Haematocrit did not differentiate innocent murmurs from abnormal murmurs.

Key words: Anaemia; Congenital; Dogs; Physiologic; Screening.

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Eur J Comp An Pract, Summer 2016; (26)1: p4-10 Go to http://www.ejcap.org for the interactive online presentation of this paper

Abbreviations:

CI	confidence interval
SD	standard deviation
MANOVA	multivariate analysis

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Cardiac auscultation is routinely performed on each pup on the first and subsequent veterinary health checks, to detect murmurs.[1,2] Although a cardiac murmur may be indicative of a congenital cardiac anomaly, a murmur may be present with no underlying heart disease, usually called an innocent murmur.[1–10] Deciding whether a murmur is innocent or the result of a cardiac anomaly could be challenging for general practitioners, as the diagnosis is based solely on auscultation.[11–15] This decision, however, is important, because a pup with a presumably pathologic murmur should ideally be referred to a veterinary cardiologist.[1,2]

Although physiologic anaemia has been described to contribute to the development of innocent murmurs in children,[8,16] to our knowledge there are no studies that have investigated this in puppies. Also, we have found no information concerning the prevalence of cardiac murmurs in clinically healthy puppies at the age group when the first veterinary health checks usually take place.[17]

The present study, thus, aims to answer the following questions. What is the prevalence of innocent murmurs in clinically healthy puppies at 2–3 months of age? Is there a correlation between the presence of an innocent cardiac murmur and a lower haematocrit?

Materials and Methods

Sub-Studies

The study was a cross-sectional survey with two separate parts. The first part, called the pilot study, was conducted over 12 months, from February 2013 until February 2014. The second part, called the principal study, was conducted over 6 months, from July 2014 until January 2015. The only difference between the pilot and the principal study was that in the principal study an echocardiogram was performed on dogs that had a cardiac murmur.

The pilot study had two research questions, namely identifying the prevalence of cardiac murmurs in clinically healthy puppies at the age when the first veterinary consults usually take place and testing for a correlation between haematocrit and the presence of cardiac murmurs. The principal study applied the same questions to innocent cardiac murmurs.

Animals

All dogs were client-owned clinically healthy puppies. Breeders of the dogs of the pilot and the principal studies participated in a voluntary screening program for congenital porto-systemic shunts. Breeders of dogs of predisposed breeds brought their litters and imported individual puppies to the clinic for individual measurement of fasted blood ammonia concentration. In the pilot study 389 puppies were enrolled and none were excluded. Eleven breeds were represented: 295 Cairn terriers (76%), 16 Yorkshire terriers, 15 Irish wolfhounds, 13 Norfolk terriers, 13 Bernese mountain dogs, ten Stabyhouns, nine West Highland white terriers, five pugs, five Scottish terriers, four Norwich terriers and four Jack Russell terriers. The age of the dogs varied from 20 to 108 (mean 53) days; the youngest Cairn terrier was 47 days old and the oldest was 80 days old.

In the principal study 210 puppies were enrolled. All but one puppy, were part of the shunt-screening project. The remaining puppy was referred to the cardiology service for evaluation of a cardiac murmur. This boerboel pup was added to the haematocrit-innocent murmur correlation analysis because it had an innocent murmur and the haematocrit value was available. Of the 210 puppies 15 were excluded from the haematocrit-innocent murmur correlation analysis. Fourteen of these (seven Irish wolfhounds, four white German shepherd dogs and three Bernese mountain dogs), actually all the large-breed puppies (except for the boerboel), were excluded because tachypnoea-induced increased respiratory sounds prevented the investigator from recognizing soft cardiac murmurs. One Cairn terrier was excluded from the haematocrit-innocent murmur correlation analysis because it had a congenital cardiac anomaly. After exclusions, 195 puppies remained for further analyses, representing seven breeds: 147 Cairn terriers (75%), 18 Jack Russell terriers, 14 Yorkshire terriers, eight Norfolk terriers, 4 Scottish terriers, three pugs and one boerboel. The age of the puppies varied from 45 to 92 (mean 54; median 53) days.

Blood Test

To measure blood ammonia concentration, approximately 2 mL of venous blood was taken via jugular venipuncture by a single experienced veterinary technician (HvE).

The sample was sent immediately to the laboratory in a vacutainer tube containing EDTA as anticoagulant. The surplus blood sample was used to measure the haematocrit using an automatic haematology analyser system[a] within 60–120 minutes. The haematocrit was measured only after all the puppies of that specific day had been auscultated, so that the investigator would not be biased.

Auscultation

The breeders gave oral permission to the single investigator (VSz, ECVIM board-certified cardiologist) to perform the cardiac auscultation, whereas they were waiting for the blood ammonia results. Each dog was identified by a chip number. The dogs were placed one by one on the examination table in a quiet examination room and were examined in standing position using a paediatric non-electronic stethoscope. The membranous side of the stethoscope with a diameter of 30 mm was used. The regions of the heart base and apex were auscultated on the left and right hemithorax. The left side was auscultated first, then the right, and finally the left side again. If a murmur was detected, the following parameters were noted: point of maximal intensity (left or right hemithorax, apex or base), intensity (scale 1–6, 6 being the loudest), place in the cardiac cycle (systolic and/or diastolic or continuous) and additional characteristics, such as musical character and beat-to-beat variability in murmur intensity (i.e. intermittently audible murmur).[4,18] A murmur was defined as intermittent if a soft (1 or 2 out of 6) murmur was heard for the first time on the left hemithorax, but disappeared while the auscultation was still performed on the same location and could not be identified when the left hemithorax was auscultated again. Auscultation of a pup lasted approximately 1 minute. Heart rates were not recorded.

Each dog was classified based on the auscultation into one of the following four categories: no murmur, suspected congenital cardiac anomaly, suspected innocent murmur and inability to judge the presence of a murmur. Dogs in the last category were excluded from further analysis. A murmur was suspected to be innocent, if it was audible intermittently or with every heart beat and had the reported characteristics of an innocent murmur: early systolic with an intensity of 1 or 2 of 6 and a musical character with the point of maximal intensity in the region of the left cardiac base.[4]

Echocardiography

Between the pilot and the principal study periods, a single Cairn terrier pup with a moderately loud (3 of 6) systolic musical murmur and four Cairn terrier pups (all from the same 50-day-old litter) with an intermittent systolic murmur were examined with an echocardiogram. The point of maximal intensity of the murmur in all the five puppies was in the region of the left cardiac base. The maximal murmur intensity in the latter four puppies was 1 of 6. In the principal study each dog with a cardiac murmur underwent a focused echocardiogram (n = 30), except for the puppies with an intermittent murmur. The focused echocardiograms[b] were performed, by the same cardiologist who did the cardiac auscultation, immediately after cardiac auscultation when the haematocrit results were not yet available. As the auscultation and the focused echocardiogram were performed by the same person, this part of the investigation was not blind. The puppies were examined in right lateral recumbency without sedation with manual restraint. Standard 2-dimensional right parasternal long axis views with colour Doppler mode were used to look for mitral, aortic and relevant tricuspid valve regurgitation, atrial and ventricular septal defects. Right parasternal short axis images were used to measure the peak blood flow velocity in the pulmonic artery with continuous wave Doppler technique to rule out pulmonic stenosis. With colour Doppler mode, the same image was used to look for a patent ductus arteriosus, atrial and ventricular septal defects and relevant tricuspid valve regurgitation. A subcostal view was used to measure the peak blood flow velocity in the aorta using continuous wave Doppler mode to rule out aortic stenosis.[5] Blood flow velocities in the pulmonic artery and in the aorta below 2.0 m/s were considered to be physiologic, whereas a blood flow velocity above 2.0 m/s would be considered to be the cause of a murmur.

Statistics

A commercially available software package[c] was used for data analysis. Because Cairn terriers outnumbered the other breeds, the findings on the Cairn terriers and those on all the other breeds were analysed separately in a subanalysis. Normality of data was assessed with the Kolmogorov–Smirnov test. A possible correlation between the presence of a cardiac murmur and the haematocrit was tested with Student's t-test. Multivariate analysis

(MANOVA) was performed to investigate whether the haematocrit or the age of the dogs correlated with the presence of (innocent) murmur, after having performed Levene's test of equality of error of variances. The P-values of <0.05 were considered to be significant. Results were described as mean (range) if the data were normally distributed. The possible correlation between the haematocrit and age of the dogs were investigated with the Pearson correlation test.

Results

Murmur

In the pilot study, 58 of the 389 clinically healthy puppies had a cardiac murmur (15%). The murmur had a musical character in 43 of the 59 cases (73%). The murmur was present only intermittently in five dogs (8%). The intensity of the murmur was 1 of 6 in 22 dogs (37%) and 2 of 6 in 31 dogs (53%).

In the principal study, 54 of the 195 clinically healthy puppies had a cardiac murmur (prevalence of 28%). This includes the one Cairn terrier where a congenital cardiac anomaly was suspected based on auscultation, which dog was subsequently excluded from the haematocrit–murmur correlation analysis, but it does not include the boerboel with an innocent murmur that was added only for the haematocrit–murmur correlation analysis. The innocent murmurs had a musical character in 49 of the 54 puppies (91%). The intensity of the innocent murmur was 1 of 6 in ten dogs (19%) and 2 of 6 in 20 dogs (37%). The murmur was only intermittently present in 24 dogs (44%), in each case with a maximal intensity of 1 of 6. The point of maximal intensity of the innocent murmurs was localized on the left hemithorax (generally in the basal region) in all but two dogs. In these two puppies the murmur was best heard in the region of the right cardiac base; one intermittently and the other one with an intensity of 2 of 6. The Cairn terrier with a suspected congenital cardiac anomaly had a systolic non-musical murmur with a stenotic character, with the point of maximal intensity in the region of the left cardiac base and an intensity of 3 of 6.

Though the heart rate was not recorded, it did not typically change remarkably during the auscultation of the individual dogs.

Echocardiography

The Cairn terrier that was examined outside the pilot and principal study periods was 65 days old and had a systolic murmur with the point of maximal intensity at the region of the left cardiac base and an intensity of 3 of 6. Echocardiography showed a mildly increased peak systolic velocity in the aorta of 2.8 m/s with no other abnormalities on the heart.

Echocardiography of the four Cairn terriers with intermittent systolic cardiac murmurs of a maximal audible murmur intensity of 1 of 6 revealed no abnormalities; the mean peak systolic blood flow velocities in the aorta and pulmonic artery were respectively 1.5 m/s (range 1.2–1.9 m/s) and 0.87 m/s (range 0.71–1.1 m/s).

In the principal study, 31 puppies underwent a focused echocardiogram. The Cairn terrier that was suspected to have a congenital cardiac anomaly based on auscultation turned out to have a double-chambered right ventricle with a maximum calculated pressure gradient of 55 mmHg. On 29 of the remaining 30 puppies, no abnormalities were found on echocardiogram. In one pup, however, a mild mitral valve regurgitation was found on colour Doppler images with no other cardiac changes. The mean peak systolic aortic flow velocity in the 30 dogs was 1.35 m/s (range 1.05–1.87 m/s) and the mean peak pulmonic arterial velocity was 0.99 m/s (range 0.74–1.26 m/s). With colour Doppler imaging, mild (physiologic) pulmonic valve regurgitations were found in twelveof the 30 puppies and mild (physiologic) tricuspid valve regurgitations in a further six.[19,20] Echocardiogram showed no abnormalities in the 69-day-old boerboel.

Haematocrit

In the pilot study, the mean haematocrit of the 389 pups was 32.0% (SD 3.1%, range 24.6–46.7%). The mean haematocrit of the group without a murmur (32.2%, SD 3.1; n = 330) was significantly (P = 0.003) higher than the mean haematocrit of the group with a murmur (30.9%, SD 2.8; n = 59). The mean age of the group without a murmur (52.9 days, SD 7.7; n = 330) was not significantly different (P = 0.2) from the mean age of the group with a murmur (51.6 days, SD 6.2; n = 59). The possible effect of breed was investigated. When

the group of Cairn terriers with a murmur (n = 53) was compared to the group of Cairn terriers without a murmur (n = 242), the same results were found as for the total group. Cairn terriers without a murmur had a mean haematocrit of 32.2% (SD 2.5%), which was significantly (P = 0.001) higher than the mean haematocrit of the group of Cairn terriers with a murmur, 30.9% (SD 2.5%). The mean age of Cairn terriers without a murmur (53.1 days, SD 6.3) was not significantly different (P = 0.47) from the mean age of the Cairn terriers with a murmur (52.4 days, SD 4.6).

However, when the mean haematocrit value of the group consisting of all non-Cairn terrier breeds (n = 94) was looked at, no statistically significant difference (P = 0.61) was found between the subgroup with a murmur (mean haematocrit 31.3%, SD 4.9; n = 6) and the subgroup with no murmur (mean haematocrit 32.2%, SD 4.4; n = 88). The mean age of the pups with and without a murmur also did not significantly differ in the non-Cairn terrier group (P = 0.07).

In the principal study group, the mean haematocrit of the 195 pups was 30.5% (SD 3.3%, range 23.2–40.8%). The mean haematocrit of the group without a murmur (30.8%, SD 3.4; n = 141) was significantly (P = 0.023) higher than the mean haematocrit of the group with a murmur (29.6%, SD 2.7; n = 54). The mean age of the group without a murmur (53.9 days, SD 7.8; n = 141) was not significantly different (P = 0.8) from the mean age of the group with a murmur (53.6 days, SD 7.6; n = 54). When the group of Cairn terriers with a murmur (n = 45) was compared to the group of Cairn terriers without a murmur (n = 102), the same results were found as for the total group. The mean haematocrit in Cairn terriers without a murmur (30.8%, SD 3.1) was significantly (P = 0.005) higher than the mean haematocrit of the group of Cairn terriers with a murmur (29.3%, SD 2.5). The mean age of Cairn terriers without a murmur (n = 102) was 53.5 days (SD 7.2 days), which was not significantly different (P = 0.49) from the mean age of the Cairn terriers with a murmur (n = 45), which was 52.7 days (SD 4.8 days).

When the mean haematocrit value of the group that consisted of all non-Cairn terrier breeds (n = 48) was considered, no statistically significant difference (P = 0.77) was found between the subgroup with a murmur (31.3%, SD 3.0; n = 9) and the subgroup with no murmur (30.9%, SD 4.3; n = 39). The mean age of the pups with a murmur (57.7 days, SD 15.2; n = 9) and those without (54.7 days, SD 9.2; n = 39) did not significantly differ in the non-Cairn terrier group either (P = 0.447).

The MANOVA showed both in the pilot and the principal study that the presence of a murmur was correlated with the haematocrit (P = 0.003 and 0.023, respectively), but not to the age of the dogs (P = 0.211 and 0.807, respectively).

A comparable, weak, but significant correlation (R = 0.339 and 0.355; P < 0.001) was found between the haematocrit and the age of the dogs in both the pilot (haematocrit = 0.247 + 0.001 [age in days]) and the principal (haematocrit = 0.224 + 0.002 [age in days]) study. The Cairn terrier puppy that was examined outside the two study periods with a mildly increased aortic flow velocity had a haematocrit of 37.0%.

Discussion

In our pilot study, 15% of 389 puppies, with the mean age of 7.5 weeks (53 days) had an audible murmur which was thought, but not proven, to be innocent.[4] In the principal study 28% of the 195 puppies with the mean age of 54 days had an innocent cardiac murmur.

In all puppies of the pilot study, the murmur was thought to be innocent based on earlier descriptions in the dog and human.[4–10] The major limitation of the pilot study is that no echocardiogram was performed on puppies with a murmur. Neither were the dogs later rechecked for spontaneous disappearance of the murmur. Although a free of charge recheck examination of all the puppies with a murmur was offered to the breeders, only a couple of puppies returned.

Cairn terriers have not been reported to be predisposed to congenital aortic or pulmonic stenosis,[14] but we identified a Cairn terrier pup with a mild aortic stenosis and a musical type of murmur. This murmur was louder than 2 of 6 (2 of 6 was the loudest innocent murmur in our principal study results). A limitation of the principal study is that dogs with an intermittently audible murmur did not undergo an echocardiogram. We thought that the auscultation and echocardiographic results of the four dogs with intermittently audible innocent murmurs examined between the two study periods could be safely extrapolated to the 24 puppies with the intermittently present soft murmur of the principal study.

It is difficult to compare the prevalence of innocent murmurs in our study to published studies, because major differences in the populations may affect findings. Innocent murmurs have been found in 58% of 10[5] clinically healthy adult whippets and in 6–12% of 95 adult dogs of different breeds.[5,17] Athletic breeds (such as whippet) are more prone to have innocent murmurs because of larger cardiac output.[5] In children, the prevalence of innocent murmurs is 50–90%.[6,10,12] The prevalence of murmurs, especially the intermittent ones, was significantly higher in the principal study (P = 0.007). The most likely explanation for this finding is the investigator's gain in experience in recognizing quickly very soft and intermittently audible murmurs.

The mean haematocrit of puppies at the mean age of 7.5 weeks was 30.5 (pilot) or 32.0% (principal study), which is lower than the reference values of adult dogs. This is consistent with physiologic anaemia, which is known to be present in young animals and children.[8,21] For example, a longitudinal study of 34 Beagles and 44 Labrador retrievers found at the age of 3.1–8 weeks a median haematocrit of 36% (29–41%) in the Beagles and a median haematocrit of 32% (20–38%) in the Labrador retrievers.[21] In the same dogs between 8.1 and 16 weeks of age, median haematocrit had risen to 38% (28–46%) for the Beagles and 37% (20–69%) for the Labrador retrievers.[21] Increasing haematocrit in growing puppies could explain why innocent murmurs tend to disappear spontaneously with aging.

In both parts of our study, puppies with cardiac murmurs had significantly lower haematocrit values than puppies with no murmur; both substudies had a low P-value. Cardiac murmurs are known to be caused by turbulent blood flow.[8,22,23] The chance of turbulent blood flow increases with the lower blood viscosity that results from lower haematocrit.[8] This relationship is described by the Reynolds number.[8,24] In addition to lower blood viscosity, a higher cardiac output has also been suggested to contribute to the presence of innocent murmurs.[8,25–27]

Although the present study has not aimed to investigate the cardiac output, higher cardiac output may be present in individuals with lower haematocrit, as anaemia has been shown to result in a hyperdynamic circulation.[28]

Blood flow velocities measured in the aorta and pulmonic artery in the puppies with an innocent murmur were comparable to the reported reference values of healthy dogs.[20,29]

The reason why no correlation was found between a lower haematocrit and murmurs in the non-Cairn terrier puppies is difficult to explain. One reason could be that the number of non-Cairn terrier dogs was quite low compared to the Cairn terrier group and thereby the power to detect a difference was too low. Another explanation could be that the non-Cairn terrier group in the pilot study included many large-breed dogs. A thicker thoracic wall may be speculated to dampen a soft murmur to an inaudible level. Another difficulty may arise from the fact that large-breed dogs are more likely to pant or to have tachypnoea. The increased respiratory sounds caused by tachypnoea might have prevented the examiner from picking up soft innocent cardiac murmurs. For this reason, large-breed dogs with tachypnoea or panting were excluded from further analysis in the principal study, but the results of the remaining small-breed dogs turned out to be the same, i.e. no correlation between haematocrit and innocent cardiac murmurs.

Because there is considerable overlap between the haematocrit values of the group with murmur and that without, measuring haematocrit would not help to differentiate an innocent murmur from a pathologic one in an individual puppy. Moreover, the difference in haematocrit between the two groups is very small. This small difference, however, seems to be large enough to create a soft murmur. Excitement may also contribute to the audibility of (intermittent) murmurs.[30]

We conclude that physiologic anaemia contributes to the genesis of innocent cardiac murmurs in puppies. Physiologic murmur can be expected in 15–25% in clinically healthy puppies brought for the first veterinary control. A murmur is likely to be innocent if it is: soft (with a maximal intensity of 2 of 6), systolic, has a musical character and the point of maximal intensity is located in the region of the left cardiac base.

Footnotes

a Advia 2120i, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany.
b Philips HD 11 XE ultrasound machine equipped with an 8– 3 MHz phased array transducer, Bothell, WA, USA.
c IBM Statistics SPSS 21.0, IBM Corp., Chicago, IL, USA.

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Conflict of Interest Declaration:

Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration:

Authors declare no off-label use of antimicrobials.

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Reprint paper*

Coagulation status in dogs with naturally occurring *Angiostrongylus vasorum* infection

Sophie Adamantos[1], Sophie Waters and Amanda Boag

SUMMARY

Objectives: *Angiostrongylus vasorum* infection is associated with bleeding tendencies in approximately one-third of clinical cases. The cause of the coagulopathy is poorly understood but may be related to disseminated intravascular coagulation. Thromboelastography is a global evaluation of coagulation and has not been described in a cohort of dogs with this disease.

Materials and Methods: Thromboelastography in association with other measures of coagulation including prothrombin and activated partial thromboplastin times, antithrombin percentage activity and D-dimer and von Willebrand factor concentrations was evaluated in a group of 30 dogs with *A. varosum infection*.

Results: A total of 18 dogs had signs of bleeding on physical examination. Thromboelastography was consistent with hypocoagulation in 17 of these dogs. There was no association between any of the other measures and hypocoagulation on thromboelastography. Abnormal coagulation times were not significantly associated with bleeding. Only fibrinogen concentration was significantly lower in dogs that were bleeding compared with those that were not (P=0.026). D-dimer concentrations were increased in 22/25 cases in the study; however, other coagulation parameters were more variable.

Clinical Significance: Although the changes identified in this study were not consistent, there is activation of coagulation within this population, possibly consistent with an intravascular disseminated coagulopathy.

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Introduction

Naturally occurring *Angiostrongylus vasorum* infection is associated with a number of clinical syndromes in dogs. Bleeding tendencies were recognised in approximately one third of cases diagnosed in England in a small case series (Chapman et al. 2004). While the coagulopathy associated with *A. vasorum* infection has been clinically recognised for some time, published information is limited to case series, case reports and experimental work (Schelling et al. 1986, Ramsey et al. 1996, Gould & McInnes 1999, Cury et al. 2002, Gallagher et al. 2012, Whitley et al. 2005). A recent study suggested that the parasite in South America may be genetically distinct from the parasite associated with natural infection in Europe, thus questioning the clinical relevance of some of this experimental work in naturally infected European cases (Jefferies et al. 2009).

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There are a number of theories postulated that could explain the pathogenesis of the bleeding diatheses seen in these patients, of which the presence of chronic disseminated intravascular coagulation (DIC) appears to be most widely accepted (Koch & Willesen 2009).

Other theories include acquired deficits in von Willebrand factor, accumulation of immune complexes stimulating the intrinsic coagulation system, immune-mediated thrombocytopenia or inhibition of coagulation because of anticoagulant factors secreted by the parasite (Caruso & Prestwood 1988, Ramsey et al. 1996, Gould & McInnes 1999, Whitley et al. 2005, O'Neill et al. 2010). All these studies have used traditional methods of laboratory assessment of haemostasis. Thromboelastography (TEG) is a newer technique that provides a global assessment of coagulation. It assesses the influence of both primary and secondary coagulation on blood clotting and can also be used to identify hypercoagulability and clot strength. TEG in dogs with DIC has been previously performed, with the most common finding being evidence of hypercoagulability (Wiinberg et al. 2008). In that study, five dogs had *A. vasorum* infection, four of which were hypercoagulable with the fifth being normal, when coagulation was assessed using TEG. It was not reported whether these dogs had signs of bleeding or not.

The aim of the current study was to describe the haemostatic abnormalities seen in dogs diagnosed with naturally occurring *A. vasorum* infection in an endemic area of England through evaluation of traditional and global coagulation tests including TEG. Further evaluation for the presence of thrombosis and fibrinolysis was also performed using markers of DIC such as increased D-dimer and low fibrinogen concentrations. As TEG is thought to be a more global assessment for bleeding tendencies than prothrombin time (PT) and activated partial thromboplastin time (aPTT), it was hypothesised that all dogs with signs of haemorrhage would have abnormalities on TEG. It was further hypothesised that dogs presenting with clinical signs of haemorrhage would have more marked abnormalities in their coagulation parameters than those that were not confirmed to be bleeding.

Finally, alterations in the coagulation panels in dogs with hypocoagulability on TEG were investigated.

Materials and methods

Dogs that were presented to the Queen Mother Hospital for Animals, Royal Veterinary College, with a diagnosis of *A. vasorum* infection were enrolled in the study. Dogs were referred for investigation and management of a variety of clinical signs and were diagnosed with *A. vasorum* infection during investigation. The diagnosis was based on identification of L3 larvae in the faeces of affected dogs using Baermann sediment evaluation.

Faecal samples were collected after voiding or rectal examination. In most cases, a single faecal sample was used for Baermann examination because of the requirement for a rapid diagnosis. However, because the result of this examination can be delayed by 24 hours, dogs were enrolled if suspicion of the disease was high and later excluded if the test was negative. Dogs were also excluded if treated with agents effective against *A. vasorum* within two weeks of presentation or immediately before testing. Owner consent was obtained for enrolment in the study. Institutional ethical approval was obtained prior to the start of the study.

Clinical information collected for each case included signalment, main presenting signs, whether there were bleeding diatheses on physical examination, post-mortem examination or magnetic resonance imaging and outcome. Blood was collected atraumatically by venipuncture of the jugular or saphenous vein and submitted for analysis on the day of admission.

TEG was performed between 30 minutes and two hours after sampling according to in-house standard operating procedures. The samples were not activated before analysis. Citrated whole blood samples were used and added to a cup containing 280 mmol $CaCl_2$ to give a total volume of 360 µL/cup. A heparinase cup was used for all samples in case of prior heparin exposure. The analyses were run for a total of 120 minutes and readings were obtained continuously by the machine during this time. Measurements obtained from the analysis included reaction time (R), clotting time (K), angle (a), maximum amplitude (MA) and global clot strength (G). Results were compared with previously established reference intervals for this machine using this technique (Goodwin et al. 2011).

Excess citrated plasma was obtained by centrifugation at 4000× g for 2 minutes; it was then separated and frozen at –30°C for coagulation profile analysis via batch submission to Comparative Coagulation Laboratory (Cornell University College of Veterinary Medicine Animal Health Diagnostic Centre, Ithaca, NY). Samples were sent frozen on ice within three months of sampling. A coagulation panel consisting of aPTT, PT, and clottable (Clauss) fibrinogen was performed using an automated clot detection instrument (STA Compact, Diagnostica Stago, Parsippany, NJ), commercial reagents (Dade Actin FS, Dade Behring, Newark, DE; Thromboplastin LI, Helena Diagnostics, Beaumont, TX; Fibrinogen, Diagnostica Stago, Parsippany, NJ) and reaction conditions as previously described (Stokol et al. 2000a). A pooled canine plasma (prepared from 20 healthy, adult dogs) was used as the fibrinogen assay standard.

The fibrinogen content of the standard was measured by gravimetric method (Gaffney & Wong 1992). Antithrombin activity (AT) was measured in a functional assay configured to measure thrombin inhibition (anti-IIa assay) using a commercial chromogenic kit (Stachrom ATIII, Diagnostica Stago, Parsippany, NJ) and the manufacturer's automated analyser. (STA Compact, Diagnostica Stago) Antithrombin activities of the test samples and plasma VWF concentration were reported as percentage of the pooled canine plasma, which had an assigned value of 100%. D-dimer concentration in ng/mL was measured using a quantitative, immunoturbidometric method as previously described (Delgado et al. 2009), using a commercial kit and the manufacturer's human D-dimer standards (HemosIL, D-dimer Calibrator, Instrumentation Laboratory, Bedford, MA). Plasma von Willebrand factor concentration [von Willebrand factor antigen (VWF:Ag)] was measured using an ELISA, configured with monoclonal anti-canine vWF antibodies (Benson et al. 1991). Fibrinogen and D-dimer concentrations have been converted to SI using standard conversion factors.

The reference intervals were provided by the laboratory performing the analysis. Automated platelet counts were performed on EDTA blood and a minimum platelet count estimate was performed on blood smear examination by a clinical pathologist. If the platelet counts could not be estimated due to clumping, the clinical pathologist made an assessment based upon a count in the body of the smear and this was defined as adequate if greater than 150×10^9/L.

Dogs were defined as having increases of PT and aPTT if they were greater than 125% of the upper end of the reference interval. Dogs were defined as being hypocoagulable if they had two or more of four of the following; increased R, increased k, decreased alpha or decreased MA, and hypercoagulable if two or more of the following were present; decreased R, decreased k, increased alpha or increased MA. Decreased G was used as a global measure of hypocoagulability and increased G as a global measure of hypercoagulability.

Statistical analysis

Data were entered into a statistical package for analysis (SPSS 21, IBM, Hampshire, UK). Data were analysed for normality graphically and using the Kolmogorov–Smirnov test and are presented as mean (±sd) when normally distributed and median (range) when not. For analysis, the data were categorised into dogs with clinical signs of bleeding and those that had not. Mann–Whitney U test was used for comparison of groups in non-parametric data and an independent sample t-test was used for parametric data. A Fisher's exact test was used for categorical data. A P value of less than 0.05 was considered significant.

RESULTS

A total of 30 dogs were enrolled in the study. All dogs had a confirmed diagnosis of *A. vasorum* infection using the

Table 1. Fibrinogen concentration, antithrombin percentage activity, D-dimer concentration and von Willebrand factor concentration in dogs that had bleeding diatheses and those that did not

Coagulation parameter	Not bleeding n=10, median (interquartile range (IQR))	Bleeding n=15, median (IQR)	P value
Fibrinogen (mmol/L)	16.02 (11.72–23.53)	3.18 (1.18–14.20)	0.026
Antithrombin (%)	87.5 (77.25–101.25)	90 (76.00–101.00)	0.935
D-dimer (nmol/L)	3.32 (1.47–4.39)	3.84 (1.82–5.44)	0.461
Von Willebrand factor (%)	185 (114.00–217.25)	110 (59.00–152.00)	0.129

Baermann method. Complete data were available for 25 cases. In five cases, TEG was performed but coagulation profile data were unavailable.

The majority of dogs were pure bred (29/30, 97%) and represented 18 different breeds with Staffordshire bull terrier (n=6) and cocker spaniel (n=4) most commonly represented. There were 8 females entire, 8 females neutered, 10 males entire and 4 males neutered. Median age was two years (range 0.3–11 years). Presenting signs included neurological abnormalities (seizures, ataxia, coma and paralysis) in 16 (53%), spontaneous non-traumatic bleeding in 10 (33%), dyspnoea in 9 (27%) and polydipsia and polyuria related to hypercalcaemia in 2 (7%) dogs. Some dogs presented with more than one sign, most commonly neurological signs and episcleral haemorrhage. On physical examination, imaging or at post-mortem examination, 18 (60%) dogs presented with bleeding diatheses. In a further three dogs, bleeding was suspected in the central nervous system, but was not confirmed, as no imaging or investigation was performed; for statistical analysis, these dogs are included in the 'not bleeding' group. In nine dogs, no bleeding was identified or suspected. A total of 21 (70%) dogs survived to discharge. Of the nine (30%) dogs that died, six had bleeding signs on presentation.

Platelet counts were available for 18 dogs. Clumps were present in eight of these blood smears, making accurate estimation impossible. In a further three dogs, the platelet count was stated to be adequate. Thrombocytopenia (platelet count less than 150 without clumps present) was confirmed in four dogs. The median (range) platelet count using the minimum estimated count was 133×10^9/L (40–342). Because of the subjective nature of these data, no further analysis has been performed.

A coagulation panel was measured in 25 dogs, of which 15 had signs of bleeding. PT and aPTT were prolonged in eight (32%) and aPTT was abnormal in a further four (16%) cases. Antithrombin was within reference interval in 24 (96%) of these cases (median 92.5% range 45–119 reference interval 65–145) and decreased in one (4%) case. D-dimer concentrations were increased in 22 (88%) cases, (median 3.35 nmol/L, range 0.27–6.30, reference interval 0–1.37). Von Willebrand's factor antigen concentration was decreased in four (16%) and increased in eight (32%) cases. Fibrinogen was measured in 18 cases and was decreased in 9 (50%) and increased in 9 (50%) cases. At least one coagulation measure was abnormal in 24 of 25 (96%) dogs and 3 or more were abnormal in 14 (56%) dogs.

Nine out of 10 dogs that did not have evidence of bleeding had normal coagulation times (PT and aPTT). Eight out of 15 dogs with bleeding diatheses had normal coagulation times. Abnormal coagulation times were not significantly associated with the presence of bleeding diatheses.

There was no difference between antithrombin percentage activity, D-dimer concentration and von Willebrand factor antigen concentrations in dogs with bleeding diatheses and those without. Fibrinogen concentration was lower in those with bleeding diatheses (median 3.18 mmol/L, range 0.44–26.31), compared with those without (median 16.02 mmol/L, range 1.32–26.02) (P=0.026). Table 1 summarises these findings. Fibrinogen concentration was also lower in dogs that had abnormal coagulation times (median 1.18mmo/L, range 0.44–1.47), compared with those that did not (median 15.99, range 3.18–26.31) (P=0.001).

TEG was performed in 30 dogs. Hypocoagulability was present in 22 (73%) dogs. Of the 18 dogs with bleeding signs, 17 were hypocoagulable on TEG. Five dogs with no

Table 2. TEG parameters in animals that were bleeding compared with those without evidence of bleeding

TEG parameter	Not bleeding n=12, median (IQR)	Bleeding n=18, median (IQR)	P value
R (minutes)	10.4 (6.88–12.73)	17.5 (12.78–22.48)	0.008
K (minutes)	3.3 (1.75–4.75)	N/A	N/A
A (angle)	40.0 (24.93–50.08)	10.0 (6.98–27.93)	0.001
MA (mm)	55.5 (46.60–59.68)	18.6 (10.25–40.28)	0.004
G dynes/s	6.2 (4.35–7.43)	6.2 (4.35–7.43)	0.004

Table 3. Fibrinogen, antithrombin, D-dimers and von Willebrand factor concentrations in dogs that were hypocoagulable on TEG and those that were not

Parameter	Hypocoagulable n=18, median (IQR)	Not hypocoagulable n=7, median (IQR)	P value
Fibrinogen (mmol/L)	4.09 (1.20–17.43)	15.99 (13.11–22.76)	0.097
Antithrombin (%)	89.0 (75.25–102.00)	90.0 (80.00–98.00)	0.745
D-dimers (nmol/L)	3.74 (1.86–5.35)	2.86 (1.63–4.44)	0.574
Von Willebrand factor (%)	117.5 (62.75–207.25)	176.0 (125.00–194.00)	0.357

evidence of bleeding were hypocoagulable on TEG, two of these dogs had neurological signs; seizures and hindlimb paralysis, with the remainder presenting for dyspnoea. Hypocoagulability identified on TEG was associated with the presence of bleeding diatheses (P=0.003).

Hypercoagulability was present in three (10%) dogs. None of these dogs had signs of bleeding. Two of these dogs were diagnosed with pulmonary hypertension. No other dogs were diagnosed with pulmonary hypertension in this study; however, echocardiography was not performed in all cases. There were significant differences identified in R (P=0.008), a (P=0.001), MA (P=0.004) and G (P=0.004) between dogs with bleeding diatheses and those without (Table 2). K time could not be analysed as in 12 dogs, the clot did not reach sufficient strength to allow its measurement. There was no difference in fibrinogen concentration, D-dimer concentration, antithrombin percentage activity or von Willebrand factor concentration between dogs that were hypocoagulable on TEG and those that were not (Table 3).

Outcome was not different between dogs that had bleeding diatheses, abnormal PT or aPTT, or were hypocoagulable on TEG analysis.

Discussion

To the authors' knowledge, this is the first study to describe some of the changes in coagulation in a large population of naturally infected dogs presenting with clinical signs of angiostrongylosis including cases both with and without bleeding diatheses. It also appears to be the first study to report on TEG findings in this group of infected cases. It should be noted that the population was a referral population and therefore likely represents a more severely affected population than that seen in first opinion practice; the changes reported therefore may not represent the changes seen in less severely affected dogs. The prevalence of bleeding in first opinion cases is likely to be lower, as reported in the study by Willesen et al. (2009). The signalment of dogs in this study is similar to that in other clinical studies (Chapman et al. 2004, Willesen et al. 2009) although the prevalence of bleeding signs is higher in the group reported here. This is likely to be related to the increased awareness of the disease over the last 10 years resulting in less severely affected dogs being managed in first opinion practice.

Laboratory assessment of coagulation parameters in this study did not include analysis of platelet count and function. Platelet counts were only submitted in 21 dogs; this reflects the fact that many of these dogs would have been admitted out of hours or at weekends and would therefore have had in-house blood smear evaluation performed. In 18 dogs, estimated platelet counts were available although in 8 of these cases, a minimum count was provided due to the presence of clumps in the sample, resulting in a pseudothrombocytopenia. In a further three samples, no count was provided, only an estimation of adequate numbers. It is likely therefore that the median platelet count presented here is an underestimation; however, mild thrombocytopenia may also be a feature of this disease as has been previously reported (Cury et al. 2002). Although it would have been preferable to have more accurate platelet estimates in a higher proportion of patients, it also seems unlikely that thrombocytopenia is the cause of coagulopathy in this disease as most dogs had a platelet count above that at which spontaneous haemorrhage is likely to occur.

A large proportion of the study population had bleeding diatheses. It is possible that some dogs included in the non-bleeding group had internal haemorrhage with no signs of external haemorrhage, which may have introduced bias. This is particularly true of the dogs presenting with neurological signs where bleeding has been identified as the cause of signs in a number of cases (Garosi et al. 2005, Wessmann et al. 2006).

Coagulation abnormalities were common in the study population, with most dogs having one or more abnormality present; however, there was no typical pattern. D-dimer concentrations were increased in 88% of dogs. Increased D-dimer concentration is associated with increased fibrinolysis as can be seen in systemic inflammation, neoplasia and following surgery. It is also present in cases of DIC (Stokol et al. 2000b). The changes in D-dimer concentration observed in this study may be a result of systemic inflammation or as a result of DIC. DIC is a complex coagulopathy associated with severe underlying diseases. It has features of both hypercoagulability and hypocoagulability resulting in both thrombosis and bleeding. DIC is a purported mechanism for the coagulopathy of *A. vasorum* (Wiinberg et al. 2008, 2010) and would explain the changes seen in fibrinogen concentration in this study. Fibrinogen concentration was significantly lower, and outside of the reference interval, in dogs that had signs of bleeding compared with those that did not. The diagnosis of DIC has not been standardised in dogs, although there is a model-based scoring system (Wiinberg et al. 2010). Application of this scoring system was not possible in this study as it used reference intervals for tests run at one specific laboratory. The current findings are suggestive of a consumptive process with activation of coagulation, consistent with DIC. It is not clear, however, whether these changes are the cause of the haemorrhage or a result of it.

Increases in fibrinogen concentration were noted in nine dogs; hyperfibrinogenaemia has not been previously reported. It is likely related to the significant inflammatory response to the parasite that occurs in the pulmonary parenchyma (Caruso & Prestwood 1988).

Routinely performed coagulation tests were abnormal in 40% of cases, and most of these dogs had signs of bleeding; however, a further eight dogs with signs of bleeding had normal coagulation tests. Hypocoagulability was identified on TEG in 22 dogs and 17 out of the 18 dogs with bleeding diatheses had hypocoagulable TEG results. Hypocoagulability was not associated with any specific abnormalities in D-dimer concentration, antithrombin percentage activity, fibrinogen concentration or von Willebrand factor concentration. As PT and aPTT were normal in some cases, it could be assumed that secondary coagulation is intact in some dogs bleeding with *A. vasorum* infection and supports a role for abnormalities in primary coagulation, including platelet function.

Platelet dysfunction is difficult to evaluate using TEG, as it represents a global evaluation of coagulation. Platelet dysfunction, however, tends to be associated with an increased k and reduced MA on TEG, although this is not specific (Bowbrick et al. 2003). Both of these were present in this population of dogs. Platelet function analysis was not performed in this group of dogs given the findings and the suggestion of dysfunction would provide a logical area for further investigation.

TEG did identify hypocoagulability in two dogs presenting with neurological disease with no other visible signs of bleeding. Although haemorrhage was not confirmed in these dogs, it seems a reasonable explanation. Given these findings, TEG does not seem to offer any clear advantage over physical examination for identification of haemorrhage; however, it may be beneficial in cases where bleeding is the suspected pathogenesis and other tests have failed to identify a cause.

Identification of hypercoagulability is a useful application of TEG and in this series, three dogs with hypercoagulability were identified. In two of these dogs, pulmonary hypertension was present, which was attributed to hypoxia and infiltration associated with angiostrongylosis. Pulmonary hypertension was not identified in any other dogs in this study; however, echocardiography was not performed and therefore the incidence of pulmonary hypertension in this population is unknown. No other dogs showed clinical signs attributable to right-sided heart failure. The relationship between hypercoagulability and pulmonary hypertension associated with angiostrongylosis would be an interesting area for further investigation as it may provide future therapeutic options.

This study provides some interesting results, but does not completely explain the cause of bleeding secondary to angiostrongylosis. Dogs presenting with bleeding diatheses have hypocoagulability on TEG, but inconsistently have alterations in secondary coagulation. D-dimer concentrations are increased and may hint at the presence of DIC. In addition, the findings suggest alterations in primary haemostasis. Further analysis of platelet function should be prioritised as an area of research in these dogs.

TEG may be useful in association with PT and aPTT in dogs presenting with bleeding in order to decide on the best use of blood products. In the presence of clinically relevant haemorrhage, alterations in PT and aPTT would provide

an indication for the use of fresh frozen plasma, whereas if these were normal with hypocoagulability on TEG, primary haemostatic dysfunction may be suspected and other therapies may be preferred. While TEG provides the clinician with a method of globally evaluating coagulation, it does not specifically evaluate platelet function and other methodologies may be more suitable for this such as multiple electrode aggregometry or advanced whole clot analysis. Because of its high sensitivity for identification of coagulopathy in this population, TEG can also be used to rule out significant coagulopathies. Although in bleeding dogs it does not seem to confer benefits in clinical practice over careful and thorough clinical examination or clinical suspicion of haemorrhage, use of TEG may be particularly beneficial for identification of hypercoagulability and may influence therapy in this group in particular.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Reprint paper*

Outcome of pyometra in female dogs and predictors of peritonitis and prolonged postoperative hospitalization in surgically treated cases

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ABSTRACT

Background: One of the most common diseases in intact bitches is pyometra– a potentially life-threatening disease associated with a variety of clinical and laboratory findings. The aims of the present study were to describe complications of the disease and to investigate clinically useful indicators associated with peritonitis and/or prolonged postoperative hospitalization.

Results: A retrospective study was performed using records from 356 bitches diagnosed with pyometra during the years 2006–2007 at the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden. Of the 356 bitches, 315 were surgically treated by ovariohysterectomy, nine were medically treated and 32 were euthanized without treatment. In the surgically treated bitches, univariable associations between clinical and laboratory data, risk for prolonged hospitalization (≥ 3 days) and/or signs of peritonitis, were analysed by Chi-square and Fisher's exact test. Logistic regression models were used to assess multivariable associations. The most common complication observed in surgically treated bitches was peritonitis (40 bitches), followed by urinary tract infection (19 bitches), wound infection (eight bitches), uveitis (six bitches), and cardiac arrhythmia (five bitches). Leukopenia and fever/hypothermia were associated with increased risk for peritonitis (18-fold and three-fold, respectively). Moderate to severe depression of the general condition, pale mucous membranes and leukopenia were associated with increased risk (seven-fold, three-fold, and over three-point-five-fold, respectively) for prolonged postoperative hospitalization.

Conclusions: Several clinically useful indicators were identified. Leukopenia was the most important marker, associated with 18-fold increased risk for peritonitis and an over three-point-five increased risk for prolonged hospitalization. Fever/hypothermia, depression and pale mucous membranes were associated with increased risk for peritonitis and/or prolonged hospitalization. The results of the present study may be valuable for identifying peritonitis and predicting increased morbidity in surgically treated bitches with pyometra.

Keywords: Bitch, Uterine inflammation, Surgical treatment, Hospitalization, Peritonitis, Risk, Outcome, Dogs

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Background

One of the most common diseases in intact bitches is pyometra affecting approximately 25% before 10 years of age [1]. Differences in incidence rates between breeds have been described [2-4]. The diagnosis is based on case history, physical examination, and laboratory analyses, often combined with radiography and/or ultrasonography of the uterus and ovaries. Clinical signs vary depending on severity of disease [5,6]. Leucocytosis, neutrophilia with left shift, anaemia, monocytosis, hypoalbuminaemia as well as affected liver or kidney function are common findings [7]. Pyometra has deadly consequences if left untreated and despite modern treatment routines the mortality is 3-4% [1]. The safest and most effective treatment is ovariohysterectomy (OHE) but purely medical treatment can be used in selected cases [8]. Though OHE is a routine procedure, anaesthesia and surgery in bitches suffering from severe systemic disease or/and organ malfunctions may be hazardous [9]. The majority of bitches with pyometra suffer from systemic inflammatory response syndrome, which previously has been associated with increased hospitalization and mortality rates [10]. It is important, but clinically difficult, to predict outcome which is why indicators for survival, complications and morbidity are wanted. Prognosis or mortality prediction by investigating different physical status and laboratory parameters is currently in demand in both human and veterinary medicine. Guidelines for performing anaesthesia and assessing anaesthetic risk based on different criteria are also being developed [11,12]. Most clinical variables are, however, unspecific, which is why current research focuses on identifying clinically valuable biomarkers with high sensitivity and specificity. In diseases with low mortality, such as pyometra, duration of postoperative hospitalization has been used as a measure for morbidity [5,13-18]. The present study explored clinical and laboratory parameters as indicators of morbidity, measured by duration of postoperative hospitalization and/or peritonitis, using analysis of multivariable associations.

Potentially life-threatening complications of pyometra, described after surgery, include sepsis, septic shock, disseminated bacterial infection, peritonitis and haemorrhage [19-21]. Clinical signs such as vomiting, diarrhoea, abdominal distention, decreased appetite or abdominal pain may be observed in animals with septic peritonitis [22,23]. These signs are, however, commonly encountered in bitches suffering from pyometra with or

Table 1 Case history, physical and laboratory examination data as recorded in 356 bitches with pyometra

Variable	In no of bitches/ total no of bitches with data recorded	Proportion of bitches with respective finding (%)
Case history		
Vaginal discharge	237/309	76.7
Anorexia	193/280	69.0
Depression	225/356	63.0
Polydipsia	180/292	61.6
Polyuria	171/288	59.4
Vomiting	75/356	21.1
Lameness	56/342	16.4
Diarrhoea	55/356	15.4
Urinary tract infection	19/342	5.6
Clinical findings		
Fever	96/301	31.9
Dehydration	94/356	26.4
Abdominal pain on palpation	81/356	22.7
Palpable enlarged uterus	67/356	18.8
Hyperaemic mucous membranes	58/356	16.3
Pale mucous membranes	52/356	14.6
Hypothermia	12/301	4.0
Laboratory analyses		
Haematology		
Anaemia	88/177	49.7
Neutrophilia	119/215	55.3
Leucocytosis	121/223	54.3
Monocytosis	108/213	50.7
Band neutrophils	31/208	14.9
Toxic neutrophils	21/223	9.4
Leukopenia	8/223	3.6
Neutropenia	8/215	3.7
Monocytopenia	7/213	3.3
Clinical chemistry		
Increased ALP	71/192	37.0
Increased bile acids	7/30	23.3
Increased lactate	2/20	10.0
Increased BUN	2/31	6.5
Increased creatinine	11/228	4.8
Hypoglycaemia	7/156	4.5
Hyperglycaemia	6/156	3.8

Normal = Within the reference range for healthy bitches and for laboratory variables at the Clinical chemistry laboratory, University Animal Hospital, Swedish University for Agricultural Sciences, Sweden. Data listed includes the number of bitches/the total number of bitches with data recorded and proportion (%) with respective finding.

without peritonitis [24]. For this purpose, researchers have studied biomarkers as indicators of severity of disease and outcome [13,25]. However, adding analyses that are not routinely performed in clinical work may be time consuming and the cost benefit must be considered. The value of clinical analyses and variables routinely available such as case history data, clinical signs, physical examination findings or laboratory biomarkers has not yet been fully explored as indicators of outcome after surgical treatment of pyometra. The aims of the present study were to describe complications of pyometra and to investigate variables that may be useful as indicators of peritonitis and/or prolonged hospitalization after surgical treatment.

Results

During the years 2006–2007, 356 bitches of 92 different breeds were diagnosed with pyometra. The age range was one to 15 years (mean ± SD, 9 ± 1.4 years). Seventy-two bitches were > 10 years old. All bitches were diagnosed within two months of the previous oestrus. Oestrus prevention by medroxyprogesterone acetate (MPA, the only registered treatment in Sweden) is rare in Sweden, and was only given to one bitch in the study material.

Case history data, physical examination and laboratory findings

Case history, physical and laboratory examination data from the 356 bitches with pyometra are shown in Table 1.

Treatment alternatives

In total, 315 bitches were surgically treated by OHE. In 65 surgically treated bitches (21%), antimicrobial therapy had been administered prior to admission, and in 124 bitches (35%) antimicrobials were administered postoperatively. Nine bitches were selected for medical treatment. Thirty-two bitches (9%) were euthanized after diagnosis without treatment.

Mortality

The total mortality was 10% (36/356) including euthanasia of 32 bitches and postoperative death of four bitches (1%) (Table 2). Of the bitches that died postoperatively, one died due to splenic rupture, one due to severe peritonitis and two of unknown causes (Table 3). Euthanasia was performed due to concomitant diseases including severe hip dysplasia (n = 1), hepatic disease associated with ascites (n = 1), long-term polyuria/polydipsia (n = 1), several other diseases (n =

Table 2 Total number, mortality, cases diagnosed with peritonitis or that had prolonged hospitalization (numbers and proportions) in bitches with pyometra that were euthanized, treated surgically or medically

Bitches	n	Mortality n (%)	Peritonitis n (%)	Prolonged hospitalization n (%)
All	356	36 (10%)	44 (12%)	60 (19%)
Euthanized	32	32 (100%)	4 (12.5%)	0 (0%)
Surgically treated	315	4 (1%)	40 (13%)	60 (19%)
Medically treated	9	0 (0%)	0 (0%)	0 (0%)

Table 3 Clinical signs, findings on physical and laboratory examinations and cause of death of the four bitches that died after surgical treatment of pyometra

Bitches	Case history	MPhysical status[a]	Laboratory analyses[b]	Cause of death
No. 1	Polyuria, polydipsia, bloody vaginal discharge, mild depression	Hypothermia, mild dehydration, CRT1–2 sec.	Leucocytosis	Unknown
No. 2	Anorexia, severe depression	Severe dehydration, abdominal pain, hyperaemic mucous membrane, CRT> 2 sec.		Severe peritonitis
No. 3	Anorexia, bloody-purulent vaginal discharge, mild depression	CRT1–2 sec.	Anaemia, increased ALP, increased lactate	Ruptured spleen
No. 4	Vomiting, diarrhoea, purulent vaginal discharge, severe depression		Leukopenia, increased ALP	Unknown

2), kidney malfunction (n = 1), mammary tumours (n = 1), multiple neoplasia in oesophagus (n = 1), or due to pyometra associated with old age of the bitch (n = 24). None of the bitches were euthanized because of a poor prognosis of pyometra.

Complications in all pyometra patients

Complications reported in the 356 bitches with pyometra were peritonitis (12.4%, n = 44), urinary tract infection (5.3%, n = 19), wound infection (2.2%, n = 8), uveitis (1.7%, n = 6), cardiac arrhythmia (1.4%, n = 5), persistent polyuria/polydipsia (0.3%, n = 1), hepatic disease associated with ascites (0.3%, n = 1) and kidney malfunction (0.3%, n = 1).

Complications in surgically treated pyometra cases

In total, there were specific complications and prolonged postoperative hospitalization of the surgically treated bitches observed in 25% (78/315) and 19% (60/ 315), respectively. The specific complications observed were peritonitis (13%, 40/315) including eight bitches with ruptured uterus, urinary tract infection (6%, 19/ 315), wound infection (3%, 8/315), uveitis (2%, 6/315), and cardiac arrhythmias (1%, 5/315).

Indicators for prolonged postoperative hospitalization and/or peritonitis

Clinical signs, physical examination findings and laboratory variables that were investigated for possible associations with prolonged hospitalization (≥ 3 days) or peritonitis are shown in Tables 4 and 5, respectively. These analyses were only performed in surgically treated bitches. The age and weight did not differ significantly between bitches with or without peritonitis or prolonged hospitalization (data not shown). Other variables not shown were not associated with peritonitis and/or prolonged hospitalization. Results of the multivariable analyses are presented in Tables 6 and 7. The Hosmer-Lemeshow goodness-of-fit statistics were not significant ($p = 0.86$ and $p = 0.71$, respectively) indicating a good fit of the multivariable models. The models explained 30 and 21% of the variation, as assessed by the generalized R^2.

Table 4 Univariable analysis of association between clinical signs, physical examination findings and laboratory data and the risk of prolonged postoperative hospitalization (≥ 3 days) in bitches with pyometra

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Case history				
Anorexia	Yes	34/167 (20)	69	0.08
	No	9/79 (11)		
Polyuria	Yes	25/151 (16)	62	0.17
	No	24/102 (23)		
Polydipsia	Yes	24/159 (15)	59	0.04
	No	25/97 (16)		
Vomiting	Yes	21/68 (31)	0	0.005
	No	39/247 (16)		
Diarrhoea	Yes	13/51 (25)	0	0.2
	No	47/264 (18)		
Vaginal discharge	Yes	44/205 (21)	0	0.005
	No	12/68 (18)		
Depression	Normal (brightness)	12/126 (9)	0	<0.0001
	Mild	20/116 (17)		
	Moderate	15/54 (28)		
	Severe	13/19 (68)		
Lameness	Yes	13/48 (27)	14	0.11
	No	44/253 (17)		
Urinary tract infection	Yes	3/19 (16)	14	0.7
	No	54/282 (19)		

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Table 4 Univariable analysis of association between clinical signs, physical examination findings and laboratory data and the risk of prolonged postoperative hospitalization (≥ 3 days) in bitches with pyometra (Continued)

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Physical examination				
Body temperature	Normal	31/175 (18)	50	0.4
	Fever	20/82 (24)		
	Hypothermia	1/8 (12)		
CRT	Normal	25/133 (19)	165	0.11
	Abnormal	6/17 (35)		
Mucous membranes	Normal	35/220 (16)	0	0.01
	Pale	14/43 (33)		
	Hyperaemic	10/51 (20)		
	Toxic	1/1 (100)		
Hydration status	Normal	37/232 (16)	0	0.004
	Mild	15/67 (22)		
	Moderate	7/15 (47)		
	Severe	1/1 (100)		
Abdominal pain	Yes	20/69 (29)	0	0.02
	No	40/246 (16)		
Palpable uterus	Yes	13/59 (22)	0	0.5
	No	47/256 (18)		
Ophthalmological exam	Ocular discharge	56/287 (19)	0	0.48
	Conjunctivitis	4/22 (18)		
	Uveitis	0/6 (0)		
Laboratory analysis				
WBC	Normal	21/90 (23)	102	< 0.0001
	Leucocytopenia	7/8 (87)		
	Leucocytosis	16/115 (14)		
Neutrophils	Normal	19/85 (22)	110	< 0.0001
	Neutropenia	7/8 (87)		
	Neutrophilia	15/112 (13)		
Band neutrophils	Normal	33/166 (20)	118	0.25
	Increased	9/31 (29)		
Toxic neutrophils	Yes	7/21 (33)	102	0.1
	No	37/192 (19)		
Monocytes	Normal	23/90 (26)	112	0.0004
	Monocytosis	14/106 (13)		
Hb	Normal	9/32 (28)	260	0.7
	Low	5/21 (24)		
	High	1/2 (50)		
Hct	Normal	15/85 (18)	148	0.38
	Anaemia	19/82 (23)		
ALP	Normal	17/113 (15)	134	0.48
	Decreased	1/4 (25)		
	Increased	14/64 (22)		
Creatinine	Normal	35/209 (17)	100	0.0009
	Increased	4/6 (67)		

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Table 4 Univariable analysis of association between clinical signs, physical examination findings and laboratory data and the risk of prolonged postoperative hospitalization (≥ 3 days) in bitches with pyometra (Continued)

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Laboratory analysis (cont.)				
Bile acids	Normal	4/23 (17)	285	0.5
	Increased	2/7 (29)		
BUN	Normal	5/26 (19)	288	0.09
	Uraemia	1/1 (100)		
Glucose	Normal	24/137 (17)	167	0.01
	Hypoglycaemia	4/6 (67)		
	Hyperglycaemia	1/5 (20)		
Lactate	Normal	4/17 (23)	296	0.03
	Increased	2/2 (100)		

a Normal = Within the reference range (as indicated below) for healthy bitches and for laboratory variables at the main Clinical chemistry laboratory, University Animal Hospital, Swedish University for Agricultural Sciences, Sweden. CRT = Capillary refill time (1–2 s), WBC = White Blood Cell Count (5.8-16.0 $\times 10^9$/L), Hb = Haemoglobin (132–199 g/L), Hct = Haematocrit (38-57%), ALP = Alkaline phosphatase (<5.0 µkat/L), BUN = Blood urea nitrogen (2.5-8.5 mmol/L), Neutrophils (3.0-11.5 $\times 10^9$/L), Monocytes (0.2-1.4 $\times 10^9$/L), Creatinine (40–130 µmol/L), Bile acids (<10 µmol/L), Glucose (4.5-5.8 mmol/L), Lactate levels (<2.2 mmol/L), Body temperature (38–39.2°C).

Table 5 Univariable analysis of associations between clinical signs, physical examination findings and laboratory data and presence of peritonitis in bitches with pyometra

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Case history				
Anorexia	Yes	22/166 (13)	70	0.05
	No	4/79 (5)		
Polyuria	Yes	17/148 (11)	65	0.34
	No	16/102 (16)		
Polydipsia	Yes	15/154 (10)	64	0.07
	No	17/97 (17)		
Vomiting	Yes	14/64 (22)	8	0.01
	No	25/243 (10)		
Diarrhoea	Yes	8/51 (16)	8	0.5
	No	31/256 (12)		
Vaginal discharge	Yes	27/203 (13)	44	0.99
	No	9/68 (13)		
Depression	Normal (brightness)	8/119 (7)	8	<0.0001
	Mild	12/115 (10)		
	Moderate	11/54 (20)		
	Severe	8/19 (42)		
Lameness	Yes	8/48 (17)	14	0.4
	No	31/253 (12)		
Urinary tract infection	Yes	1/19 (5)	14	0.3
	No	38/282 (13)		

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Table 5 Univariable analysis of associations between clinical signs, physical examination findings and laboratory data and presence of peritonitis in bitches with pyometra (continued)

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Physical examination				
Body temperature	Normal	16/170 (9)	57	0.006
	Fever	19/81 (23)		
	Hypothermia	0/7 (0)		
CRT	Normal	15/133 (11)	166	0.03
	Abnormal	5/16 (31)		
Mucous membranes	Normal	22/213 (10)	8	0.02
	Pale	8/42 (19)		
	Hyperaemic	8/51 (16)		
Hydration status	Normal	25/224 (11)	8	0.004
	Mild	8/67 (12)		
	Moderate	5/15 (33)		
	Severe	1/1 (100)		
Abdominal pain	Yes	14/67 (21)	8	0.02
	No	25/240 (10)		
Palpable uterus	Yes	6/58 (10)	8	0.5
	No	33/249 (18)		
Ophthalmological exam	Ocular discharge	37/279 (13)	8	0.5
	Conjunctivitis	2/22 (9)		
	Uveitis	0/6 (0)		
Laboratory analysis				
WBC	Normal	10/90 (11)	102	< 0.0001
	Leucocytopenia	3/5 (62)		
	Leucocytosis	11/115 (10)		
Neutrophils	Normal	9/85 (11)	110	< 0.0001
	Neutropenia	3/5 (62)		
	Neutrophilia	10/112 (9)		
Band neutrophils	Yes	18/166 (11)	118	0.07
	Increased	7/31 (23)		
Toxic neutrophils	Yes	4/21 (19)	102	0.3
	No	22/192 (11)		
Monocytes	Normal	12/90 (13)	112	0.02
	Monocytopenia	3/7 (43)		
	Monocytosis	9/106 (8)		
Hb	Normal	5/32 (16)	260	0.5
	Decreased	4/21 (19)		
	Increased	1/2 (50)		
Hct	Normal	10/85 (12)	148	0.9
	Anaemia	9/82 (11)		
ALP	Normal	14/108 (13)	141	0.48
	Decreased	0/4 (0)		
	Increased	5/62 (8)		
Creatinine	Normal	21/202 (10)	107	0.01
	Increased	1/5 (20)		

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Table 5 Univariable analysis of associations between clinical signs, physical examination findings and laboratory data and presence of peritonitis in bitches with pyometra (continued)

Variable[a]		No. of bitches with prolonged hospitalization/bitches with variable n (%)	Missing data	p-value (Chi-square test/ Fisher's exact test)
Laboratory analysis (cont.)				
Bile acids	Normal	3/23 (13)	285	0.9
	Increased	1/7 (14)		
BUN	Normal	5/26 (19)	285	0.63
	Uraemia	0/1 (0)		
Glucose	Normal	16/133 (12)	173	0.05
	Hypoglycaemia	2/4 (50)		
	Hyperglycaemia	0/5 (0)		
Lactate	Normal	4/17 (23)	296	0.4
	Increased	1/2 (50)		

a Normal = Within the reference range (as indicated below) for healthy bitches and for laboratory variables at the Clinical chemistry laboratory, University Animal Hospital, Swedish University for Agricultural Sciences, Sweden. CRT = Capillary refill time (1–2 s), WBC = White Blood Cell Count (5.8-16.0 $\times 10^9$/L), Hb = Haemoglobin (132–199 g/L), Hct = Haematocrit (38-57%), ALP = Alkaline phosphatase (<5 µkat/L), BUN = Blood urea nitrogen (2.5-8.5 mmol/L), Neutrophils (3.0-11.5 $\times 10^9$/L), Monocytes (0.2-1.4 $\times 10^9$/L), Creatinine (40–130 µmol/L), Bile acids (<10 µmol/L), Glucose (4.5-5.8 mmol/L,), Lactate levels (<2.2 mmol/L), Body temperature (38–39°C).

Table 6 Multivariable logistic regression model of association between clinical signs, physical examination findings and laboratory data and the risk of prolonged postoperative hospitalization (≥ 3 days) in bitches with pyometra (n = 184)

Variable[a]		Estimated coefficient	Odds Ratio (95% confidence interval)	p-value
Polydipsia[b]	Yes	–0.83 (±0.44)	0.43 (0.18 - 1.02)	0.056
Vomiting[b]	Yes	0.77 (±0.46)	2.16 (0.87 - 5.38)	0.097
Depression	Mild	1.14 (±0.57)	3.14 (1.02 - 9.64)	0.008
	Moderate to severe	1.91 (±0.61)	6.78 (2.03 - 22.59)	
Mucous membranes	Pale	1.13 (±1.37)	3.09 (1.06 - 8.96)	0.021
	Hyperaemic	–0.95 (±0.64)	0.39 (0.11 - 1.35)	
WBC	Leukopenia	1.26 (±1.37)	3.53 (0.24 - 51.73)	0.012
	Leucocytosis	–1.31 (±0.49)	0.27 (0.1 - 0.71)	

a Normal = Within the reference range for healthy bitches and for laboratory variables at the main Clinical chemistry laboratory, University Animal Hospital, Swedish University for Agricultural Sciences, Sweden; WBC = White blood cell count (ref. range 5.8-16 $\times 10^9$/L); b The variable is not statistically significant ($p > 0.05$), but included as a confounder in the model.

Table 7 Multivariable logistic regression model of associations between clinical signs, physical examination findings and laboratory data and the presence of peritonitis in bitches with pyometra (n = 158)

Variable[a]		Estimated coefficient	Odds Ratio (95% confidence interval)	p-value
Polydipsia[b]	Yes	–0.91 (±0.53)	0.40 (0.14 - 1.13)	0.084
Body temperature	Fever/hypothermia	1.19 (±0.50)	3.30 (1.23 - 8.82)	0.017
WBC	Leukopenia	2.90 (±1.20)	18.11 (1.74 - 188.92)	0.043
	Leucocytosis	–0.10 (±0.54)	0.90 (0.32 - 2.56)	

a Normal = Within the reference range for healthy bitches and for laboratory variables at the main Clinical chemistry laboratory, University Animal Hospital, Swedish University for Agricultural Sciences, Sweden; WBC = White blood cell count (ref. range 5.8-16 $\times 10^9$/L); Body temperature (ref. range 38-39°C); b The variable is not statistically significant ($p > 0.05$), but included as a confounder in the model.

Discussion

Identifying complications in bitches with pyometra is vital for selecting optimal monitoring routines and treatments and for determination of prognosis. In the present study, complications were observed in 25% of the bitches treated by OHE. Peritonitis was the most common complication and can be life-threatening[26]. Urinary tract infection (UTI) was the second most common complication (6%). Previously, subclinical UTI with the same bacterial strain as in the uterus has been demonstrated in 25% of bitches with pyometra [27]. Though subclinical UTI may resolve without intervention after OHE, proteinuria or clinical signs of disease should be monitored to prevent severe renal disease from developing [28]. In the present study, uveitis was diagnosed in six bitches. Uveitis has not previously been associated with pyometra but has been described in dogs and cats suffering from severe bacterial infection [29,30].

Cardiac arrhythmia, as identified in five bitches, could have been induced by endotoxaemia or myocardial injury [21,31]. Peritonitis, uveitis and cardiac arrhythmias are serious, but treatable, consequences of endotoxaemia and sepsis, further supporting the importance for predicting such complications early [32-34].

In the present study, the total mortality including euthanasia was 10%, which is higher than earlier reported [1]. Our previous study included bitches < 10 years whereas the present study incorporated data from bitches of all ages, hence the 20% that were > 10 years may contribute to some extent to explain this difference since the owners could be more likely to choose elective euthanasia instead of treatment in an older dog or that has concurrent diseases (which is more likely in older dogs). Four bitches died postoperatively, resulting in a mortality of 1% after surgery, which is lower than the 5-27% in previous reports [19,31,35]. Surgical treatment of pyometra had thus a very good prognosis, when performed in the selected cases, in our study. Thirty-two (9%) bitches were euthanized instead of treated due to old age and/or concurrent diseases (such as mammary tumour), all by request of the owner and in agreement with the veterinary surgeon in charge. These bitches were excluded from the analysis of predictors for increased postoperative hospitalization, peritonitis and mortality because they were not surgically treated and their death was not associated with a poor prognosis or severity of pyometra. Age and weight did not differ between the groups (without complications and hospitalization < 3 days compared to with complications and/or prolonged hospitalization), showing that peritonitis or prolonged hospitalization was not more common in older bitches. This indicates that age by itself is not a risk factor for surgical treatment of pyometra [9].

Overall, the most common signs of disease, present in > 50% of the bitches, included vaginal discharge, anorexia, depression, polydipsia, and polyuria (Table 1), reflecting systemic involvement of the disease in the majority of bitches [19,36,37]. Vaginal discharge, which has been associated with more severe disease and is considered a characteristic sign of pyometra [6], was absent in 23% of the cases. Obscure signs of illness may make the disease more difficult to recognize and supports the need for diagnostic indicators for pyometra. Interestingly, lameness was present in 56 (16%) of the bitches, which has not been reported previously. It is possible that the activated immune response could, for example, trigger arthritis, as has been suggested in humans [38]. Regarding laboratory findings, leucocytosis with neutrophilia and left shift, monocytosis and anaemia were observed in the majority of bitches, as is common in the disease [19,39].

One of 40 bitches with peritonitis died, resulting in a 3% mortality in pyometra with peritonitis which is comparatively low compared with mortality rates of 50% previously reported due to uterine rupture [26]. Importantly, none of the bitches with ruptured uterus died. We may speculate that this could depend on the disease being common in Sweden and possible complications recognized earlier compared with in countries where the majority of the bitches are neutered.

All surgically treated bitches were included in the analyses for identifying indicators for peritonitis and/or prolonged postoperative hospitalization. Two bitches were diagnosed with peritonitis postoperatively and since it could have been subclinical at the time of surgery or caused by uterine leakage during surgery, these bitches were included in the peritonitis group when analysing for indicators. Moderate to severe depression and pale mucous membranes were associated with increased risk of prolonged postoperative hospitalization (Table 6). A striking result was that leukopenia was associated with a three-point-five-fold increased risk of having prolonged hospitalization compared to normal WBC and

also an 18-fold increased risk of peritonitis. These results make leukopenia the most important clinical biomarker identified. Leukopenia could be caused by endotoxin-induced bone marrow depression in combination with more chronic inflammatory disease and loss of leucocytes to the uterine lumen. Increased mortality has also been demonstrated in animals and humans with leukopenia in other studies [40-42]. A WBC within the normal reference range was associated with increased risk for prolonged hospitalization and/or peritonitis as compared to leucocytosis. This could possibly reflect a transition from leucocytosis to leukopenia i.e. leucocytosis appearing earlier in the pathogenesis. Not only may the number of leucocytes be decreased in pyometra, but their function (phagocytic capacity and mitogen-driven lymphocyte proliferation) is also impaired, negatively affecting the combat against infection [43,44]. Bitches with moderately to severely depressed general condition had a seven-fold increased risk for prolonged hospitalization. Pale mucous membranes, which might reflect anaemia, were associated with a three-fold increased risk of prolonged hospitalization. In contrast, hyperaemic mucous membranes were associated with decreased risk for prolonged hospitalization. Lactate levels, though only analysed in 19 dogs, were associated with increased risk for prolonged hospitalization, indicating a predictive value.

Other variables than leukopenia were linked with presence of peritonitis. Fever, or hypothermia present in merely a third of the pyometra cases, was associated with a three-fold increased risk of peritonitis indicating a prognostic value for this variable. Uterine diameter was not associated with peritonitis or prolonged hospitalization, which would otherwise be plausible since a larger uterus could have indicated more severe local disease.

Some parameters were analysed only in a few bitches hence missing data is a limitation in our study.

Retrospectively collected data may also be less reliable than prospectively collected data. Because the bitches selected for medical treatment were already less severely affected by their disease than those subjected to surgical treatment, the data reported here cannot serve as a comparison of the two treatment methods. The results of the present study will increase the possibilities to predict prognosis and outcome after surgical treatment of pyometra. This will be valuable in early identification of cases with peritonitis and/or increased morbidity (hospitalization), which will in turn aid in treatment selection and thereby possibly also increase survival.

Conclusions

Complications such as peritonitis, uveitis, urinary tract infection, wound infection and cardiac arrhythmias were observed in bitches with pyometra. Several routine parameters that may be useful as indicators of peritonitis and/or prolonged hospitalization were identified. Leukopenia was associated with increased risk for peritonitis and prolonged hospitalization making leukopenia the most important biomarker to be aware of clinically. The results of the present study will be clinically valuable for identifying peritonitis in bitches with pyometra and the prediction of prolonged postoperative hospitalization after surgical treatment.

Methods

Animals

Only journal data already available was used for the study and ethical approval therefore not necessary to obtain according to Swedish regulations.

A retrospective study was carried out using data records from all bitches diagnosed with pyometra during the years 2006–2007 at the University Animal Hospital (UDS), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Bitches were identified by the diagnostic code for pyometra used in Sweden [26]. The animal hospital's patient records include data such as breed, weight, age, case history, physical examination findings, results of radiographic and/or ultrasonographic examinations, laboratory analyses including haematology and serum biochemistry, treatments, date of dismiss ion and follow-ups at the UDS. The preliminary diagnosis pyometra was based on the results of case history, physical examination and diagnostic imaging. The cases were admitted mainly within two months of previous oestrus (in metoestrus), not associated to parturition or pregnancy and all dogs had signs of systemic illness.

Ultrasonography or radiography or both were used to demonstrate an enlarged, fluid-filled uterus. The diagnosis pyometra was verified visually during ovariohysterectomy and according to the previous definitions by De Bosschere and others (2001) [45]. Bitches with cystic endometrial hyperplasia, mucometra, hydrometra, haematometra and

endometritis were not included. The bitches diagnosed with pyometra were divided into three groups depending on whether they were euthanized, medically or surgically treated. Euthanasia was performed at the request of the owner and in agreement with the veterinary surgeon in charge due to concomitant diseases. Bitches with normal hydration status, unaffected or slightly depressed general condition and with no ovarian or endometrial cysts demonstrated on ultrasonographic examination were selected for medical treatment with aglepristone (Alizin vet®, Virbac, France) in combination with antimicrobials. The success of the medical treatment was evaluated by ultrasonography and laboratory tests including haematology, total white blood cell counts and differential counts to monitor the treatment response. In this study, all medically treated cases recovered as judged by normal laboratory tests and no uterine or ovarian pathology on diagnostic imaging. Furthermore, data from the surgically treated bitches were analysed for indicators of pre-existing peritonitis or development of postoperative peritonitis or prolonged postoperative hospitalization. Intraocular pressure was measured in all bitches with uveitis.

Variables as indicators of peritonitis and/or prolonged hospitalization

Variables included in the analyses for indicators of peritonitis and/or prolonged postoperative hospitalization were as follows: appetite, body temperature, depression, mucous membrane appearance, hydration status, capillary refill time (CRT), polyuria, polydipsia, vomiting, diarrhoea, vaginal discharge, lameness, urinary tract infection, presence of abdominal pain on palpation, palpable enlarged uterus and all other pathological findings noted when performing a complete physical examination. The following laboratory variables were included in the analyses: total white blood cell count (WBC) with differential counts and morphology, haemoglobin (Hb), haematocrit (Hct), alkaline phosphatase (ALP), creatinine, bile acids, blood urea nitrogen (BUN), glucose and lactate concentrations. Additionally, antimicrobial administration, ligation material used during surgery, presence of peritonitis at surgery, administered drugs (anaesthetic agents, analgesics, i.v. fluid therapy), duration of postoperative hospitalization and uterine diameter (as determined by ultrasonography or macroscopically during surgery) were integrated in the analyses. The study defined abnormality of haematology and blood chemistry by using the reference ranges at the main clinical chemistry laboratory, UDS, SLU. Case history, laboratory and clinical examination variables recorded before surgery were used in the analysis.

Determination of prolonged hospitalization and peritonitis

In general, bitches subjected to OHE due to pyometra at UDS are hospitalized 1–2 days. Prolonged postoperative hospitalization (defined as ≥ 3 days) is only warranted if specific complications occur, if the general condition is depressed and the bitch requires additional veterinary care and monitoring (considered as an unspecific complication).

Peritonitis was identified by free fluid and/or hyperechoic fat tissue (steatitis) detected on ultrasonographic examination of the abdomen or decreased serosal detail observed on radiographic examination (as a consequence of steatitis and/or intra-abdominal fluid). Macroscopically peritonitis was identified visually by fibrin or other signs of inflammation on the surface of the abdominal structures or pus present in the abdominal cavity or by positive bacterial culture from the abdominal fluid. In two bitches, peritonitis was diagnosed the day after surgery.

Statistical analysis

Univariable associations between potential risk factors within case history, physical examination and laboratory data and the outcomes prolonged hospitalization and signs of peritonitis, respectively, were analysed by Chi-Square test and Fisher's exact test. Multivariable associations between these potential risk factors and the outcomes were analysed by logistic regression models. All variables with a p-value ≤ 0.20 in the univariable analyses were considered as potential predictor variables. Categorical predictor variables were introduced in the models coded as dummy variables. Collinearity between potential predictor variables were assessed by variance inflation factors (VIF) above 10 [46] in which case the variable with a) least missing values or b) providing the best model fit was retained. Modelling was done manually by backward elimination of non-significant ($p > 0.05$) variables. At each step, previously eliminated variables were tested for re-entry. Confounding was assessed by comparing the change in estimated coefficients when variables were excluded from the model, and were considered present if a coefficient changed > 20%. The fit of the final multivariable model was assessed with a Hosmer-Lemeshow goodness-of-fit test [47] and the coefficient of determination was assessed with a

generalized R^2 as suggested by Nagelkerke (1991) [48]. All statistical analyses were performed using SAS (version 9.3, SAS Institute Inc., Cary, NC, USA).

Competing interests

None of the authors have any conflict of interest to declare.

Authors' contributions

SJ drafted the manuscript. CAB, SJ, RH, AP, and OH provided data and managed the data records. UE performed statistical analyses. RH, AP, BSH, CAB, UE and OH reviewed and commented the manuscript during its preparation. All authors read and approved the final manuscript.

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Reprint paper*

Cohort Study of the Success of Controlled Weight Loss Programs for Obese Dogs

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ABSTRACT

Background: Most weight loss studies in obese dogs assess rate and percentage of weight loss in the first 2–3 months, rather than the likelihood of successfully reaching target weight.

Objective: To determine outcome of controlled weight loss programs for obese dogs, and to determine the factors associated with successful completion.

Animals: 143 obese dogs undergoing a controlled weight loss program.

Methods: This was a cohort study of obese dogs attending a referral weight management clinic. Dogs were studied during their period of weight loss and cases classified according to outcome as "completed" (reached target weight), "euthanized" (was euthanized before reaching target weight), or "stopped prematurely" (program stopped early for other reasons). Factors associated with successful completion were assessed using simple and multiple logistic regression.

Results: 87/143 dogs [61%] completed their weight loss program, eleven [8%] died or were euthanized, and the remaining 45 [32%] stopped prematurely. Reasons for dogs stopping prematurely included inability to contact owner, refusal to comply with weight management advice, or development of another illness. Successful weight loss was positively associated with a faster rate ($P < 0.001$), a longer duration ($P < 0.001$) and feeding a dried weight management diet ($P = 0.010$), but negatively associated with starting body fat ($P < 0.001$) and use of dirlotapide ($P = 0.0046$).

Conclusions and Clinical Relevance: Just over half of all obese dogs on a controlled weight loss program reach their target weight. Future studies should better clarify reasons for success in individual cases and also the role of factors such as activity and behavioural modification.

Key words: Caloric restriction; Canine; Outcomes; Overweight.

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The medical profession classifies human obesity as a disease,[1] and it is arguably the most important medical disease in dogs.[2] Recent studies have suggested that approximately half of all pet dogs are overweight[3,4] and that the prevalence has been steadily increasing.[5] Obesity is associated with many diseases, including orthopaedic disease, diabetes mellitus, respiratory disease and certain types of neoplasia.[2,3] Dogs that are overweight might also develop metabolic derangements,[6,7] altered renal function,[8] and respiratory dysfunction causing poorer oxygenation.[9] Obese dogs have a reduced quality of life,[10] and a shorter lifespan.[11] Given the large at-risk population and the effects on health and quality of life, obesity is a major welfare concern. Management usually involves controlled weight loss through energy restriction using a purpose-formulated weight loss diet coupled with increased activity,[12–15] but licensed drug therapies are also available.[16,17]

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Abbreviations:

AAFCO	Association of American Feed Control Officials
AF	as fed
BMD	Bernese mountain dog
CKCS	Cavalier King Charles spaniel
CI	confidence intervals
DEXA	dual-energy X-ray absorptiometry
DM	dry matter
EBT	English Bull Terrier
F	female
FCR	Flat coated retriever
FOS	fructo-oligo saccharides
GR	Golden retriever
GSD	German shepherd dog
HPHF	high protein high fibre
HPMF	high protein medium fibre
M	male
ME	metabolizable energy
MER	maintenance energy requirement
NF	neutered female
NM	neutered male
OR	odds ratio
SBT	StaffordshirecBull Terrier
STROBE	Strengthening and reporting of observational studies in epidemiology
TDF	total dietary fibre
YT	Yorkshire Terrier

The benefits of controlled weight loss in obese dogs are well established, with evidence of improvement in disease status,[18] reversal of metabolic derangements,[6,7] and improved quality of life.[10] However, studies are often of short duration, only assessing the initial phase of weight loss (e.g. first 2–3 months) and often use colony dogs with experimentally induced obesity rather than client-owned dogs with naturally occurring disease.[16–18] As a result, simple outcomes are studied such as rate of weight loss, percentage weight loss and energy intake required to achieve weight loss.[13,14] Arguably, studies that assess the whole of the weight loss period and beyond are more desirable and also focus outcomes such as success of reaching and maintaining target weight.[19] Human studies suggest that weight loss usually plateaus at 6 months on diet-based weight loss program, with most people never reaching their target weight,[20] or subsequently regaining a substantial amount within oneyear.[21] To the authors' knowledge, only one previous study of dogs has reported success of a weight loss program,[15] although the weight loss period was short (6 months) and it was not clear whether all dogs had reached their target weight. In light of the limited information, the aims of the current study were, first, to determine the proportion of obese dogs commencing a diet-based weight loss program that successfully reached target weight and, second, to identify factors associated with success.

Methods

Study Design

This was a cohort study of obese client-owned dogs designed to determine the outcome of controlled weight loss programs and the factors associated with successful completion. It has been reported according to the Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines.[22]

Animals

The dogs in the cohort studied were all referred to the Royal Canin Weight Management Clinic, University of Liverpool UK, for investigation and management of obesity. Eligible cases were: originally seen between November 2004 and July 2012, started a weight management program and reached a known end-point for their weight loss (i.e. completed, stopped prematurely, or died [see below]) by February 2013. Additional eligibility criteria included having data available from the preliminary investigations undertaken before weight loss (see below) and having had body composition analysis conducted by dual-energy X-ray absorptiometry (DEXA).[23] Given the study timeframe and broad eligibility criteria, dogs used in previous studies assessing weight loss in selected cases that successfully lost weight only,[13,14] and also in a study examining subsequent rebound after successful weight loss.[19] However, none of these studies examined the proportion of cases starting a weight loss program that successfully reached target weight. The study protocol adhered to the University of Liverpool Animal Ethics Guidelines and was approved by the University of Liverpool Research Ethics committee, the Royal Canin ethical review committee and the WALTHAM ethical review committee. Owners of all participating animals gave informed consent in writing.

Weight Loss Regimen

Complete details regarding the weight loss protocol used at the clinic have been described.[13,14] Briefly, dogs

were determined to be systemically well and without abnormalities that would make controlled weight loss inappropriate by complete blood count, serum biochemical analysis and urinalysis. Serum free thyroxine concentration was measured by equilibrium dialysis at an accredited external laboratory[a] to determine thyroid status. Throughout weight loss, dogs were weighed on electronic scales,[b] which were regularly calibrated using certified test weights.[c] Body composition was analysed before and after the weight loss regime in all dogs, using fan-beam DEXA.[d] Body composition results from before weight loss were used to estimate ideal weight.[10,19] Briefly, the body composition data were entered into a computer spreadsheet,[e] containing a purpose- created mathematical formula to predict expected body composition after weight loss at different weights. The predictive equation was based upon typical body composition results from previous weight clinic studies.[13,14]

This enabled an appropriate ideal body weight to be set, for the individual dog, to be used in energy intake calculations. One of three purpose-formulated weight management diets was used for the weight loss protocol (Table 1), namely a high protein high fibre dry diet (HPHF dry),[f] a high protein medium fibre dry diet (HPMF dry),[g] and a high protein medium fibre wet diet (HPMF wet).[h] The choice of whether to feed dry food, wet food or a mix of the two depended upon what the owner had fed the dog before the weight loss period. Owner and dog preference was also used when choosing between the HPMF and HPHF dry diets (e.g. whether high fibre diets had been tolerated in the past). However, diet choice also depended upon availability and whether any reformulations had occurred. In this respect, both HPMF diets (dry and wet) were available for the whole of the study period and the formulation did not change. However, the HPHF diet first became available in June 2006, and was then reformulated in 2010 with a slight increase in moisture content, without major changes in the nutrient profile (Table 1), with 22 dogs of the 88 dogs fed this diet receiving the reformulated version. The ME content of both formulations was marginally different (before reformulation: 2900 kcal/ kg; after reformulation: 2865 kcal/kg).

The initial food allocation for weight loss was determined by first estimating maintenance energy requirement (MER = 440 kJ [105 kcal] 9 body weight [kg]$^{0.75}$/d)[24] using the ideal weight of the dog, as determined by DEXA. The degree of restriction for each dog was then individualized based upon gender and other factors (i.e. presence of associated diseases) and was typically between 50–65% of MER at target weight. Owners also received tailored advice on lifestyle and activity alterations to assist in weight loss. Further, five dogs whose weight loss had been slow also received oral dirlotapide[i] to aid weight loss, whereas four additional dogs had concurrent hypothyroidism (two diagnosed at the referring veterinarian and two diagnosed at the time of initial referral) and also received levothyroxine. Dogs were reweighed every 7–28 days and changes made to the weight loss plan if necessary.[13,14] Throughout the weight loss period, owners maintained a diary in which they recorded feeding of the purpose-formulated diet (amount

Table 1. Average composition of diets for weight loss.

Criterion	High protein high fibre dry[1]		High protein medium fibre dry		High protein medium fibre wet	
ME content	2900/2865 kcal/kg		3275 kcal/kg		548 kcal/kg	
	Per 100 g AF	g/1000 kcal (ME)	Per 100 g AF	g/1000 kcal (ME)	Per 100 g AF	g/1000 kcal (ME)
Moisture	8/10	28/33	8	27	86	1569
Crude protein	30/30	103/105	34	104	7.0	128
Crude fat	10/10	33/33	10	30	2.0	36
Starch	19/18	66/61	22	66	2.1	38
NFE	30/29	102/100	32	97	2.5	46
Crude fibre	18/16	60/58	8	25	1.0	18
Total dietary fibre	28/28	97/97	18	56	1.4	26
Ash	5.3/5.7	18/20	8.1	25	1.5	27
Fibre sources	Cellulose, beet pulp, FOS, psyllium husk, diet cereals		Cellulose, beet pulp, diet cereals		Beet pulp, cassia gum, carrageenan	

High protein high fibre (Satiety Support Canine, Royal Canin). High protein medium fibre (Obesity Management Canine, Royal Canin).
ME, Metabolizable energy content, as measured by animal trials according to the American Association of Feed Control Officials protocol (AAFCO, 2010); AF, as fed; DM, dry matter; FOS, fructo-oligo-saccharides; NFE, nitrogen-free extract.
[1] Diet formulation changed in 2010; figures in column refer to diets used before and after 2010, respectively.

offered and consumed) and any additional food that had been consumed (either given as treats or stolen). At each re-evaluation, progress was assessed and changes were made to the weight loss plan, as necessary. Where progress was good (e.g. weight loss of 0.5–2.0% per week in the first six months and >0.3%/ week thereafter), the weight loss protocol was not adjusted, except that the owner was always encouraged to increase activity whenever possible. If weight loss was deemed to have stalled (defined as either no change [0%] in weight or a gain of weight between two appointments that were at least 14 days apart) or was deemed to be slow (<0.5%/week in the first six months and <0.3% week thereafter), the potential causes were investigated based upon the information provided by the owner in diary records and discussions during the consultation. If poor compliance to the weight loss protocol was thought to be the cause, (i.e. additional food had been consumed) the amount of food fed was not altered and advice was given to restore compliance; if the dog's activity levels had been less, then advice regarding activity was reiterated; however, where no obvious reason for poor progress could be identified, the amount of food fed was reduced by a readily calculated amount (e.g. 5 g dry food for small dog or 10 g for large dog; ¼ sachet of wet food for a small dog or ¼ x 400 g can for large dog) on each occasion. When weight loss was deemed to be too quick (>2%/week) the amount of diet was increased in similar increments. In addition to the official reweighs, contact was maintained at other times either by phone or email.

Classification of Final Outcome

Dogs were assigned to three groups, according to their outcome, as follows. Dogs that lost weight and reached their target were classified as "'completed." Dogs that were euthanized before reaching target weight were classified as "euthanized," and the reason was recorded where it was known. Finally, dogs that did not complete for other reasons were classified as "stopped prematurely," and again the reason was recorded where it was known. This latter category included all dogs lost to follow-up because their owners stopped attending the clinic. In such cases, owners were contacted at least three times by their preferred method of contact (telephone or email) and at least once by post.

Statistical Analysis

All data are expressed as median (range), except where indicated, and there were no missing data. Statistical analyses were performed with computer software,[j] with the level of significance set at $P < 0.05$ for two-sided analyses. Given that this was an observational cohort study, and no such study had previously been conducted, a sample size calculation was not performed. Instead, the principle determinant of sample size was the number of dogs seen that met the eligibility criteria during the study timeframe. The Shapiro-Wilk test was used to determine whether or not datasets were normally distributed, and either parametric or nonparametric tests were then performed as appropriate. For continuous variables, differences among groups were assessed with the Kruskal-Wallis test, with post hoc comparisons made, where appropriate, using the Dwass-Steel-Critchlow-Fligner test.

The continuous variables analysed by groups were age, body fat percentage before weight loss, percentage weight loss, duration of weight loss, rate of weight loss, metabolizable energy intake during weight loss, the number of times weight loss stalled (i.e. when there was no change in weight or weight gain between appointments) and the number of diet energy intake changes (i.e. when the weight management clinic staff adjusted down the daily food intake at the time of a recheck). Overall percentage weight loss and rate of weight loss were both expressed as a proportion of starting weight lost and reported rates of weight loss are the average of the whole weight loss period. Duration of weight loss was calculated from the date of the first appointment to the date when target weight was reached (for those completing), or to the last available weight record (for those not completing). Where dogs were enrolled but then did not return for any reassessments, the duration was recorded as 0 days.

Categorical variables were compared, among dogs with different outcomes, using Fisher's exact test and those assessed included breed, sex, neuter status, diet characteristics, concurrent hypothyroidism and use of dirlotapide. The effect of breed was determined by first creating dummy variables for all breeds with more than five individuals (where 1 = dog of that breed; 0 = dog not of that breed). For sex comparisons, a dummy variable was created whereby male dogs were scored as 1 and female dogs as 0; a dummy variable was also created for neuter status whereby neutered dogs were scored as 1 and intact dogs as 0. The effect of diet was assessed in two ways: first, a dummy variable was created whereby comparing dogs fed dry food exclusively (including both those on HPHF and HPMF diets) were assigned a score of 1, to those fed either wet food exclusively, or a combination of dry and wet food

were assigned a score of 2; second, where dogs were fed dry food exclusively, the type of dry food was also compared (1 = HPHF diet; 0 = HPMF).

In order to take account of possible confounding factors on the results obtained, logistic regression was performed. The outcome variable tested was success with weight loss, whereby dogs completing weight loss were assigned a score of 1, and those not completing were assigned a score of 0. Both 'intention-to-treat' (whereby dogs that were euthanized were included in the group not completing), and 'per-protocol' (whereby dogs that were euthanized were excluded) analyses were conducted. Initially, all variables listed above were tested separately with simple logistic regression. A multiple logistic model was then built, which initially included the variables identified as $P < 0.2$ in simple regression. The model was then refined over multiple rounds using backward-stepwise elimination, of the least significant variable each time, and variables were only retained in the final model if they were significant ($P < 0.05$). Logistic regression results are reported as odds ratios (OR), 95% confidence intervals (95% CI) and the associated P value.

Table 2 Baseline variables of the study dogs.

Variable	Completed (n = 87)	Stopped prematurely (n = 45)	Euthanized (n = 11)	P value[4]
Breed [1]	Labrador 21 mixed breed 14 CKCS 9 Golden retriever 7 Yorkshire Terrier 7 OTHER: Alaskan Malamute, Akita, BMD, Border Collie 3, Cairn Terrier 2, Chihuahua, Cocker Spaniel 2, Corgi, Dachshund, Doberman 2, EBT, FCR, GSD, Irish Setter, JRT, Lhasa Apso, Miniature schnauzer, Pug 4, Samoyed, Schipperke, Shih Tzu	Labrador 14 mixed breed 4 CKCS 2 Golden retriever 1 Yorkshire Terrier 1 OTHER: Akita, Border Collie, Dachshund 3, Dalmatian 2, English Pointer, GSD, JRT 2, Labradoodle, Lancashire Heeler, Lhasa Apso 2, Patterdale Terrier, Poodle, Pug, Rottweiler, Scottish Terrier, Springer spaniel 2, Tibetan Terrier	Labrador 0 mixed breed 1 CKCS 1 Golden retriever 0 Yorkshire Terrier 0 OTHER: Bichon Frise, Boxer English Bulldog, EBT, Lhasa Apso, Newfoundland, SBT, Shih Tzu, Weimaraner	Lab: 0.088 Mix: 0.34 CKCS: 0.49 GR: 0.41 YT: 0.41
Reason for stopping or euthanasia	--	Personal reasons of owner 9, refused help shortly after enrolment 5, repeated failure to comply with program 3, owner chose to stop 7, dog developed another disease (pneumonia) 1, not recorded (could not contact owner) 20	Developed another disease 6 (severe orthopaedic disease, metastatic mast cell tumour, splenic neoplasia, laryngeal neoplasia, and concurrent cardiac and renal disease), not recorded 5	
Sex [2]	M 2; NM 47, F 2, NF 36	M 1; NM 25; F 2; NF 17	M 1; NM 5; NF 5	0.76
Age (Mo)	72 (16–228)[a]	84 (24–156)[ab]	96 (55–144)[b]	0.059
Start Weight (kg)	32.0 (5.3–77.6)	33.9 (4.4–60.8)	27.1 (7.2–100.0)	0.75
Body fat (%)[3]	44.8 (27.3–55.0)	46.2 (27.9–60.8)	44.2 (35.3–55.5)	0.10

All data (except diet data) are expressed as median (range).

1 Breed acronyms are as follows: BMD, Bernese mountain dog; CKCS, Cavalier King Charles Spaniel; EBT, English bull Terrier; FCR, Flat Coated Retriever; GSD, German Shepherd Dog; JRT, Jack Russell Terrier; SBT, Staffordshire Bull Terrier.

2 Sex acronyms are as follows: M, male; NM, Neutered male; F, female; NF, neutered female.

3 Body fat percentage was determined before weight loss using dual-energy X-ray absorptiometry.

4 For breed and sex, P values are based upon Fisher's exact tests (Lab: Labrador, Mix: mixed breed, CKCS: Cavalier King Charles Spaniel; YT: Yorkshire Terrier); for age, start weight and body fat, P values are based upon Kruskal-Wallis tests. Groups with different letters are significantly different from one another, at $P < 0.05$.

Results

Study Animals and Outcomes of Weight Loss

During the period of study, 160 dogs were referred to the clinic. Of these, 143 met the eligibility criterion of having a defined endpoint and there were no missing data for any variable. The other 17 dogs were excluded because the weight loss period had not been completed at the time of data review. Of the 143 dogs, 87 (61%) completed, eleven (8%) were euthanized (by the referring veterinarian) and 45 (31%) stopped prematurely. Full details of all dogs finally included are given in Table 2. There were no differences in the proportions of the five most frequent breeds among groups ($P > 0.05$ for all) and no differences in sex ($P = 0.57$), starting weight ($P = 0.75$) and body fat mass ($P = 0.16$). However, age was different among groups ($P = 0.045$), with dogs that were euthanized being older than those that completed the weight loss protocol. Three of the hypothyroid dogs completed the weight loss protocol, with the other dog stopping prematurely.

Outcomes of Weight Loss

Details of the outcomes of weight loss are reported in Table 3. For the whole cohort, percentage weight loss was 19.5% (range -3.0% to 43.9%), median duration was 200 days (range 0–1149 days) and the corresponding rate of weight loss was 0.6% per week (-0.3 to 2.2% per week).

Comparison of Baseline Variables among Groups

Comparisons were made among the three outcome groups (e.g. completed, euthanized and stopped prematurely) for all baseline variables (Table 2). There were no differences in the proportions of the five most frequent breeds among groups ($P > 0.05$ for all) and no differences in sex ($P = 0.57$), starting weight ($P = 0.75$) and body fat mass ($P = 0.16$). However, age was different among groups ($P = 0.045$), with dogs that were euthanized being older than those that completed the weight program.

Comparison of Weight Loss Outcomes among Groups

Comparisons were made among the three outcome groups (e.g. completed, euthanized and stopped prematurely) for all weight loss (Table 3). There were no differences in the median daily energy intake (per kg metabolic body weight) among groups ($P = 0.67$) and also no differences in the number of times weight loss process stalled ($P = 0.37$), the number of times food intake had to be reduced ($P = 0.16$) and the use of dirlotapide ($P = 0.082$). However, dogs that

Table 3. Outcomes of weight loss.

Variable	Completed (n = 87)	Stopped (n = 45)	Euthanized (n = 11)	P value[2]
Diet (number of dogs)[1]	HPHF dry 58[a] HPMF dry 27 HPMF wet 0 Mixed 2	HPHF dry 24[ab] HPMF dry 17 HPMF wet 2 Mixed 2	HPHF dry 6[b] HPMF dry 2 HPMF wet 2 Mixed 1	HPMF v HPHF:0.54 Dry v Wet/ mixed:0.0077
Weight loss (% start weight)	25.5 (5.5 to 43.9)[a]	8.8 (-3.0 to 33.0)[b]	16.7 (-2.3 to 39.5)[b]	<0.001
Rate of Weight loss (%/wk)	0.7 (0.1 to 1.7)[a]	0.4 (-0.3 to 2.2)[b]	0.6 (-0.1 to 1.3)[ab]	0.001
Duration (days)	250 (84 to 796)[a]	139 (0 to 1149)[b]	141 (47 to 371)[b]	<0.001
Energy intake (kcal/kg$^{0.75}$ ideal weight/d)	62.3 (44.0 to 92.9)	63.5 (42.3 to 87.1)	60.8 (51.8 to 75.2)	0.67
Weight loss stalls[3]	1 (0–6)	1 (0–18)	0 (0–6)	0.37
Diet energy intake changes[4]	2 (0–11)	2 (0–13)	2 (0–5)	0.16
Concurrent hypothyroidism	3	1	0	0.78
Dirlotapide	1	4	0	0.082

All data (except diet data) are expressed as median (range).

1 Diet types were as follows: HPHF dry, high protein high fibre dry: HPMF dry, high protein medium fibre dry, HPMF wet, high protein medium fibre wet; Mixed, mixed ration with more than one type (e.g. completed: HPHF dry with HPMF wet [n = 2]; stopped prematurely: HPMF dry and wet [n = 2]; died: HPHF dry with HPMF wet [n = 1]). Energy intake expressed in kcal of metabolizable energy per kilogram of metabolic body weight of ideal weight (kg$^{0.75}$).

2 For diet, P values are based upon Fisher's exact tests; for all other data, P values are based upon Kruskal–Wallis tests. Groups with different letters are significantly different from one another, at $P < 0.05$.

3 Number of times the weight loss process stalled.

4 Number of times food intake had to be reduced.

succeeded remained on their weight loss program longer ($P < 0.001$), had faster overall rates of weight loss ($P = 0.001$) and lost more weight overall ($P < 0.001$). An effect of diet type was also seen, with more of the completing dogs having been fed dry food than either wet food or a mix of types ($P = 0.0077$). However, there were no group differences in the type of dry food used (i.e. HPHF versus HPMF diets, $P = 0.54$).

Logistic Regression Analysis to Determine Factors Associated with Success

Given that a number of group differences were evident, logistic regression analysis was then used to determine factors associated with success, when taking account of any possible confounding. When assessed on an intention-to-treat basis, simple logistic regression (Table 4) identified that rate of weight loss ($P = 0.0092$), duration of weight loss ($P = 0.014$) and diet type ($P = 0.028$) were positively associated with success, whereas starting body fat was negatively associated with success ($P = 0.029$). Other factors were not significantly associated with weight loss, but qualified (at $P < 0.2$) for inclusion in the initial multiple regression model including: age, breed (with mixed breed, Golden Retriever and Yorkshire Terrier included independently), dirlotapide use, number of weight loss stalls and number of changes to the weight loss plan (Table 4). After the initial model was refined by backward-stepwise elimination, the best-fit model was one that included six

Table 4. Results of the logistic regression analysis determining factors associated with success or failure.

Criterion	Intention-to-treat			Per protocol		
Logistic regression	**OR**	**95% CI**	**Prob-ability**	**OR**	**95% CI**	**Prob-ability**
Simple regression						
Age (per month)	0.99	0.98–1.00	0.13	0.99	0.985–1.005	0.34
Target Body Weight (per kg)	1.00	0.98–1.03	0.78	1.00	0.98–1.03	0.79
Body Fat (per %)	0.94	0.89–0.99	0.029	0.94	0.89–1.00	0.047
Breed						
CKCS	2.04	0.53–7.88	0.30	2.48	0.51–12.00	0.26
Labrador retriever	0.95	0.44–2.08	0.91	0.70	0.32–1.57	0.39
mixed breed	2.49	0.78–8.00	0.12	2.79	0.73–9.89	0.12
Golden Retriever	4.81	0.58–40.22	0.15	3.85	0.46–32.31	0.21
Yorkshire Terrier	4.81	0.58–40.22	0.15	3.99	0.46–32.31	0.21
Sex (male versus female)	0.97	0.49–1.90	0.92	0.94	0.46–1.95	0.87
Neuter Status (neutered versus intact)	1.60	0.38–6.66	0.52	1.48	0.32–6.93	0.62
Diet						
HPHF v HPMF	1.36	0.65–2.83	0.41	1.52	0.70–3.29	0.29
Dry v wet/mix	6.07	1.21–30.38	0.028	4.15	0.73–23.57	0.11
Concurrent hypothyroidism	1.96	0.20–19.37	0.56	1.57	0.16–15.55	0.70
Dirlotapide use	0.15	0.02–1.40	0.095	0.12	0.01–1.10	0.061
Rate of weight loss (per %/wk)	3.35	1.35–8.30	0.0092	4.15	1.50–11.44	0.0061
Duration (per day)	1.003	1.000–1.004	0.014	1.002	1.000–1.004	0.046
Energy intake	0.99	0.95–1.03	0.49	0.98	0.94–1.02	0.37
Weight loss stalls (per stall)	0.90	0.78–1.05	0.17	0.87	0.74–1.02	0.092
Diet energy intake changes (per change)	1.11	0.95–1.29	0.19	1.08	0.93–1.27	0.31
Multiple regression						
Breed: mixed breed	6.22	1.10–35.30	0.039	—	—	—
Body Fat (per %)	0.87	0.80–0.94	<0.001	0.88	0.81–0.96	0.0039
Diet: Dry v wet/mix	15.93	1.97–128.91	0.0095	15.37	1.57–150.71	0.019
Dirlotapide use	0.01	0.00–0.27	0.0031	0.02	0.00–0.43	0.011
Rate of weight loss (per %/wk)	10.66	2.99–38.00	<.0001	9.52	2.58–35.16	<0.001
Duration (per day)	1.010	1.01–1.013	<0.001	1.009	1.005–1.013	<0.001

OR, Odds Ratio; CI, Confidence Interval; CKCS, Cavalier King Charles Spaniel; HPMF, High Protein Medium Fibre (Satiety) diet; HPHF, High Protein High Fibre (Obesity dry diet).

factors. Factors positively associated with success included being of mixed breed (P = 0.039), being fed a dry weight loss diet (P = 0.0095), rate of weight loss (a faster rate of weight loss in completing dogs, P < 0.001) and duration (a longer duration or weight loss in completing dogs, P < 0.001), whereas factors negatively associated with success included starting percentage body fat (P < 0.001) and dirlotapide use (P = 0.0046). When data were instead analysed on a per-protocol basis by excluding dogs that were euthanized, results were similar, except that the breed effect was no longer evident (Table 4). Given that dogs fed wet food or a mix of food types were less successful, there was a concern such a categorization might have inadvertently selected for dogs with problematic feeding habits, since this category included those where diet type had been changed.

As a result, the analyses were repeated only to include dogs that had remained on the same diet type for the whole of weight loss. Once again, a diet effect remained (simple regression: OR 10.41, 95%-CI 1.22–89.00, P = 0.032; multiple regression: OR 32.50, 95%-CI 2.02– 458.68, P = 0.016).

Discussion

This large study assesses the success of obese dogs at completing a controlled weight loss program and at reaching target body weight. The finding that 40% of dogs stopped prematurely is similar to a previously published study,[15] and suggests that controlled weight loss is challenging. However, although somewhat disappointing, this response rate is better than for humans who use diet-based strategies for losing weight where few individuals succeed with weight loss.[20] The weight loss period is only one aspect of the overall weight management process, which also includes maintaining weight long term and avoiding rebound. The fact that this aspect was not assessed in the current study is a limitation, although the population studied did include cases that also participated in a previous study that did specifically assess maintenance of weight in the post-weight loss period.[19]

The large cohort size meant that we could also determine factors associated with success: associations were found with starting body fat percentage, overall rate of weight loss, duration of weight loss and the type of food used. Given that the study was observational in nature, the reasons for such associations are not always clear and causality cannot necessarily be assumed, i.e. that the factors identify cause the dogs to complete or stop prematurely. Direct associations are more likely when associations are identified with factors present at the outset of the controlled weight loss program, such as body fat mass. Here, it is reasonable to speculate that the negative association between starting body fat mass and the outcome of weight loss might be causally related and to suggest that the most overweight dogs might struggle to reach target weight. Indeed, this finding is similar to human studies where weight loss plateaus over time,[20] and is not surprising given the metabolic changes that occur upon caloric restriction.[21] In contrast, where the associations identified were with factors not present at the outset, conclusions should be more speculative. For instance, successful weight loss was positively associated with the duration of the weight loss program, and this is most likely to be because the weight loss process was curtailed in cases that stopped prematurely or were euthanized. Therefore, a long duration is a characteristic of the successful case, rather than the cause of it.

Nonetheless, while care should rightfully be taken when drawing any conclusions from these associations, these observations are still of interest since they might help to develop hypotheses to test in future studies.

A faster rate of weight loss was also positively associated with success. At first, this observation seems counterintuitive, since faster rates of weight loss should make the weight loss program shorter yet, as stated above, duration was longer in cases that successfully completed. However, the findings can readily be explained by the fact that these associations with duration and rate of weight loss were independent of one another in the final multiple regression model. The faster weight loss rate could be a characteristic of the cases that successfully lose weight, but a causal relationship might exist. In this respect, those owners whose dogs lost weight more rapidly could be motivated to persist with the program for longer, thus improving the likelihood of successfully reaching target weight. Conversely, slow weight loss progress could cause owner frustration making them more likely to stop prematurely. Of course, while such a hypothesis is intriguing, it does not explain why the dogs that stopped prematurely had a slower rate of weight loss in the first place. Possible causes might include lack of compliance with the weight loss program, difference in activity levels, or might be related to the speed of weight gain and development of obesity. A further limitation of this study was that physical activity

was not objectively assessed. Moreover, while owners were always questioned at the first consultation about the speed and duration of weight gain, most were unable to provide any detailed insight into this (for instance because weight had been infrequently recorded). Further work is required to determine their respective roles of exercise and speed of weight gain on the success of a subsequent weight loss program.

The study also identified an association between food type and successful weight loss, with a greater proportion of cases fed dry food completing than those on wet food or a mix of wet a dry food. However, the finding should be interpreted cautiously, in light of the fact that only nine dogs were fed wet food or a mixture. One possible explanation for the effect would be differences in macronutrient content of the various diets. Indeed, previous work has indicated that voluntary food intake is less when dogs are fed diets with increased protein and fibre content,[25] and such diets also promote greater fat loss during the weight loss period.[14] However, in the current study, the fact that there was no difference in success for dogs on the HPHF and HPMF foods suggests that differences in fibre content were not responsible. Thus, other reasons are likely to account for the positive association between feeding dry food and completing a controlled weight loss program. An alternative possibility would be the fact that some of the dogs on a mixed feeding combination had switched rations during their program, i.e. from dry to wet (or a mix) and vice versa. Whereas the reason for switching strategies was not recorded, it was often because of problems with progress, so that we might have inadvertently selected for less successful dogs. In light of this, we repeated the multiple regression analysis excluding dogs that had switched food type, and the effect of dry food on weight loss outcome remained. Thus, such a selection bias cannot account for effect of food type. A third possibility might be that feeding dry food affords greater control than wet food; the amount of food can be measured out precisely on weigh scales, small adjustments to the amount fed can easily be made and the food readily lends itself to methods of feeding that promote environmental enrichment, such as the use of puzzle feeders. Such feeders have been shown to slow food intake in dogs,[k] thereby improving satiety with the resulting effect of decreased food-seeking behaviour. Finally, owner factors might also explain this association, whereby the ease of using dry food might have increased compliance, thereby indirectly improving outcome. The added cost of wet food might have been an additional disincentive for owners using this format to continue with the weight loss program. Given the multiple possibilities, further studies are now required both to confirm and to determine reason for the association between diet type and successful weight loss.

Another factor that was negatively associated with the completing the weight loss program was use of the microsomal transfer protein inhibitor dirlotapide. Conclusions should be made cautiously because only a small number of dogs received the drug and it was administered in conjunction with the current weight loss diet, which is not specifically recommended. Although all foods used had 10% fat content (on an as fed basis) and previous studies have suggested a good response to dirlotapide in dogs fed food with an equivalent fat content, dogs were not fed ad libitum.[16] This might account for the negative association between dirlotapide use and successful weight loss.

Alternatively, selection bias could have been responsible, since the drug was used when cases were struggling with a conventional program using dietary caloric restriction. Nonetheless, the finding suggests that drug therapy does not always provide an additional advantage over dietary energy restriction alone in cases struggling to lose weight. Further work is required to understand better the reasons for failure of dirlotapide in the cases in which it was used.

Hypothyroidism is associated with obesity in dogs,[3] and four cases in the current series were diagnosed with this disease. We chose to include these dogs so as to ensure that our cases were as representative as possible of the obese pet dog population from which they were drawn. Including such cases in the study is a limitation because it introduces a possible confounder, for example if response to a controlled weight loss program differs from that of euthyroid obese dogs. Therefore, we would recommend further work examining the response of hypothyroid dogs to controlled weight loss.

Breed was associated with outcome of weight loss in the intention-to-treat analysis, with a greater proportion of mixed breed dogs completed compared with pedigree dogs. If genuine, it might either suggest potential genetic influences on the success of weight loss programs, or be related to owner factors (for instance, if the characteristics of a mixed breed dog owner differed from those of a pedigree dog owner). This breed effect was the weakest of all associations identified and was no longer evident when data were analysed on a per-protocol basis. Conclusions

should be even more cautious because of the limited range of breeds included, as well as the limited numbers of each breed. Therefore, further work is needed to confirm this observation before investigating the possible reasons for it further.

A number of limitations should be considered in addition to those discussed above. First, the use of a cohort design means that the basis for our findings is not clear. Thus, further studies are now needed to confirm these findings and to examine possible mechanisms. Second, the dogs studied were referred to a weight management clinic and, as a result, the findings might not be fully representative of dogs in primary care practice. Third, the use of client-owned, rather than colony, dogs introduced a number of possible confounding variables, both dog and owner related. Dog-specific factors increasing population variability include signalment factors, tendency to scavenge, ability to exercise and the presence of concurrent disease; owner-specific factors include compliance with the weight management advice on feeding and exercise. In human weight loss studies, non-compliance is common and is a major cause of treatment failure.[26] Although the use of client-owned dogs could have affected the reliability of the results, the findings are arguably more representative of the target population, such that they are more generalizable than findings from studies in colony dogs.

Finally, although numerous factors were considered, the roles lifestyle and activity alterations (including exercise) or behavioural manipulation were not examined. Advice on activity and behaviour was given to all clients, which was specific to the circumstances of the owner and the dog. Unfortunately, the nature of the advice made it impossible to assign meaningful categories for analysis. As a result of this limitation, future studies should now be considered to assess the role of both activity and behavioural modifications on the outcomes of controlled weight loss.

Conclusions

In summary, this study demonstrates approximately one half of all obese dogs on a controlled weight loss program reach their target weight. Associated with success was starting body fat percentage, with the most obese dogs less likely to reach their target weight. Since activity and behavioural modification were not specifically assessed in this study, future studies should also be considered specifically to examine their role.

Footnotes

a Axiom Veterinary Laboratories Ltd, Newton Abbott, Devon, UK
b Soehnle Professional, Backnang, Baden-Württemberg, Germany
c Blake and Boughton Ltd, Thetford, Norfolk, UK
d Lunar Prodigy Advance; GE Lunar, Madison, WI
e Excel®, various versions; Microsoft Corporation. Redmond, WA
f Canine Veterinary Diet Satiety Dry, Royal Canin, Aimargues, France
g Canine Veterinary Diet Obesity Management Dry, Royal Canin, Aimargues, France
h Canine Veterinary Diet Obesity Management Wet, Royal Canin, Aimargues, France
i Slentrol, Zoetis UK, London, UK
j Stats Direct version 2.6.8, Stats Direct Ltd
k German AJ, Towlson E, Holden SL, et al. Long-term follow-up after weight management in obese cats. Proceedings of the 55th British Small Animal Veterinary Association Congress, Birmingham, UK; April 2012

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Conflict of Interest Declaration.
AJG's Readership is funded by Royal Canin; AJG has also received financial remuneration and gifts for providing educational material, speaking at conferences and consultancy work; SLH's post at the University of Liverpool is also funded by Royal Canin; the diet used in this study is manufactured by Royal Canin; YQ and VB are employed by Royal Canin; PM is an employee of Mars Petcare.
Off-label Antimicrobial Declaration.
Authors declare no off-label use of antimicrobials.

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Reprint paper*

Predicting Outcome in dogs with Primary Immune-Mediated Haemolytic Anaemia: Results of a Multicentre Case Registry

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ABSTRACT

Background: Outcome prediction in dogs with immune-mediated haemolytic anaemia (IMHA) is challenging and few prognostic indicators have been consistently identified.

Objectives: An online case registry was initiated to: prospectively survey canine IMHA presentation and management in the British Isles; evaluate two previously reported illness severity scores, Canine Haemolytic Anaemia Score (CHAOS) and Tokyo and to identify independent prognostic markers.

Animals: Data from 276 dogs with primary IMHA across 10 referral centres were collected between 2008 and 2012.

Methods: Outcome prediction by previously reported illness-severity scores was tested using univariate logistic regression. Independent predictors of death in hospital or by 30 days after admission were identified using multivariable logistic regression.

Results: Purebreds represented 89.1% dogs (n = 246). Immunosuppressive medications were administered to 88.4% dogs (n = 244), 76.1% (n = 210) received antithrombotics and 74.3% (n = 205) received packed red blood cells. Seventy-four per cent of dogs (n = 205) were discharged from hospital and 67.7% (n = 187) were alive 30 days after admission. Two dogs were lost to follow-up at 30 days. In univariate analyses CHAOS was associated with death in hospital and death within 30 days. Tokyo score was not associated with either outcome measure. A model containing SIRS classification, ASA classification, ALT, bilirubin, urea and creatinine predicting outcome at discharge was accurate in 82% of cases. ASA classification, bilirubin, urea and creatinine were independently associated with death in hospital or by 30 days.

Conclusions and clinical importance: Markers of kidney function, bilirubin concentration and ASA classification are independently associated with outcome in dogs with IMHA. Validation of this score in an unrelated population is now warranted.

Key words: Canine haemolytic anaemia objective score; Immune-mediated haemolytic anaemia; Survival; Thromboembolism.

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Abbreviations:

ALP	alkaline phosphatase activity	IMHA	immune-mediated haemolytic anaemia
ALT	alanine aminotransferase	IQR	interquartile range
ASA	American Society of Anesthesiologists	MMF	mycophenolate mofetil
AUROC	area under the receiver-operating curve	OR	odds ratio
CHAOS	canine haemolytic anaemia objective score	ROC	receiver-operating curve
CI	confidence interval	SE	standard error
DEA	dog erythrocyte antigen	SIRS	systemic inflammatory response syndrome
hIVIG	human intravenous immunoglobulin	UFH	unfractionated heparin

Immune-mediated haemolytic anaemia (IMHA) is among the most common autoimmune condition affecting dogs,[1] and some aspects of its pathogenesis have been well characterized.[2,3] Despite such insights, the prognosis for dogs with IMHA remains guarded, with published case fatality rates for primary IMHA in dogs ranging from 26% to 60%.[4–6] Previous studies have linked various clinico-pathologic abnormalities with outcome in dogs with IMHA. Few prognostic indicators are consistent across multiple studies, however, perhaps because of differences between study populations or because of a lack of standardization.

It has been suggested that validation and standardization of diagnostic criteria is urgently required for dogs with IMHA and that future interventional clinical trials would benefit from stratification by mortality risk.[7] Mortality risk assessment for clinical trials is typically performed using illness severity scores.[8] Accurate prognostication in a complex disease process like IMHA might require a multifaceted scoring system. Two such schemes, the canine haemolytic anaemia objective score (CHAOS) and a score developed in Japan (Tokyo) have been proposed, [a 9] but neither has been independently evaluated to determine if they remain prognostic outside of the populations from which they were generated.

Alternatives to these disease-specific illness severity scores that might be easier to estimate are the American Society of Anesthesiologists (ASA) health classification and the presence or absence of markers of a systemic inflammatory response syndrome (SIRS). The ASA classification is typically used to evaluate patient risk for anesthesia,[10] but the classification is easy to apply and has been used as a marker of disease severity in other canine populations.[11] The inflammatory response associated with IMHA in dogs is well-recognized and can be evaluated through measurement of acute phase proteins,[12] or cytokine concentrations.[13] These measures are not widely available however, while a SIRS score based on readily obtained clinical data is a more universal means to identify dogs with systemic inflammation.[14]

Several studies from individual centres in the United Kingdom have been published recently, but each described relatively few cases and studied distinct aspects of the disease. Even with the benefit of these data, it is difficult to summarize the demographics, therapies and outcomes of the overall UK canine IMHA population presenting to referral centers.[15–17]

In this study we aimed to address these knowledge gaps by surveying case presentations, management strategies and outcomes of dogs with IMHA presenting to multiple referral centres in the British Isles. In addition, we aimed to test the association of illness severity markers ASA and SIRS status with outcome and test the predictive ability of two previously published IMHA-specific illness severity scores. We also aimed to identify independent prognostic markers from our own dataset using a multivariate analysis approach and hypothesized that a multivariable scoring system would predict survival better than individual variables alone.

Materials and Methods

Sample Size

Based on previous publications we estimated case fatality at discharge at 17%,[16] and that 20% dogs would have previously identified risk factors.[6,18,19] We aimed to detect a 2-fold increase in case fatality risk where such factors were present and therefore planned to enrol 335 dogs.[b]

Case Recruitment

Collaborators were recruited by publication of a letter

inviting participation,20 and through direct contact with referral centres. Data sharing was agreed in writing. Cases admitted from January 1, 2008 to December 31, 2009 were included retrospectively. Dogs admitted between January 1, 2010 and December 31, 2012 were enrolled prospectively. Dogs with primary, idiopathic IMHA admitted to participating institutions within the study period were eligible for inclusion. To maximize recruitment, the following previously published diagnostic criteria were used:[13,16] anaemia (PCV<37%) AND at least one of the following: positive in-saline agglutination test, OR a positive Coombs' test, OR moderate marked spherocytosis identified by a board-certified clinical pathologist. Dogs were excluded if evidence of a predisposing disease process was present.[21] All dogs underwent diagnostic evaluation according to their individual case histories as judged appropriate by their attending primary clinicians. These diagnostic evaluations (summarized in Table S1, see end of paper for link) were not standardized, but aimed to identify potential underlying causes and typically included CBC, serum biochemistry, thoracic and abdominal imaging, PCR testing for tick-borne infections by Babesia, Ehrlichia and Mycoplasma species and urine culture. Attending clinicians determined case management.

Data Acquisition and Handling

Study data (Data S1, see end of paper for link) were collected using secure, web-based software that enabled automated data export.c Historical, demographic, at-admission clinicopathologic, treatment and outcome data were recorded via a custom survey, accessible from January 1, 2010 to February 1, 2013, agreed in advance by all participating centres (Data S2, see end of paper for link). The survey used dropdown menus, limited-response questions and constrained textboxes to minimize errors. Free-text boxes enabled addition of contextual comments to aid interpretation. Raw data were regularly inspected and where necessary, centres were contacted to correct erroneous or incomplete entries. ASA status was assigned as follows: Grade 1, Normal; Grade 2, Mild systemic disease; Grade 3, Severe systemic disease; Grade 4, Life-threatening systemic disease; Grade 5, Moribund patient, not expected to survive.[22] Illness severity scores CHAOS,[a] and Tokyo,[9] were calculated as previously reported (Table 1). Systemic inflammatory response syndrome (SIRS) was diagnosed using published criteria: Temperature ≤100.0°F (37.8°C) or ≥103.5°F (39.7 °C); heart rate >160 bpm, RR >40 bpm; leukocyte count ≤4,000/µl or ≥12,000/µl or ≥10% band neutrophils.[23] Anisocytosis and polychromasia were graded

Table 1. Calculation of CHAOS and Tokyo illness severity scores.

Canine hemolytic anemic objective score (CHAOS)	
Age (year)	If ≥7 score 2, otherwise score 0
Temperature (°F)	If ≥102.0 score 1, otherwise score 0
Agglutination	If present score 1, otherwise score 0
Albumin (g/dL)	If <3.0 score 1, otherwise score 0
Bilirubin (mg/dL)	If ≥5.0 score 2, otherwise score 0
Total	Maximum score 7

Tokyo score	
Sex	Male score 1, Female score 0
Season	Apr-Sept score 1, Oct-Mar score 0
Packed cell volume (%)	If <20 score 1, otherwise score 0
Platelet count (x 10^3/µL)	If <200 score 1, otherwise score 0
Total protein (g/dL)	If <6.0 score 1, otherwise score 0
Total	Maximum score 5

as mild, moderate, or severe as previously described. Similarly, spherocytes were quantified in the monolayer using a 1+ to 3+ scale, where 1+ equals 5–10 spherocytes per 100 x oil field (2–4% of the RBCs); 2+ equals 11–50 (4–20%); and 3+ equals 51–150 spherocytes per field (20–60%).[21] Where discrepancies between automated and manual platelet counts occurred, manual counts were used for calculations. Saline agglutination tests were performed using a drop of EDTA-anticoagulated blood mixed with a drop of saline on a microscope slide and examined against a white background over a period of 1–2 minutes for gross agglutination, followed by microscopic evaluation for differentiation from rouleaux.

Data Analysis

Exported data were collated and analysed using proprietary software.[d,e,f] Data were assessed for normality prior to test selection. Although some variables were parametric, most were not, thus variables are reported as median (interquartile range). To validate use of the whole dataset for outcome analyses, retrospective and prospective data were compared using nonparametric tests. To correct for multiple (m) comparisons while minimizing the risk of dismissing significant differences, the P-value was adjusted: $P_{adjusted} = [0.05 \times (m+1)] / (2m)$.[24] CHAOS, Tokyo, case, treatment and centre variables were tested for association with death during hospitalization and death by 30 days by univariate logistic regression. The effect of centre was assessed using multiple dichotomous variables with a reference group.

Multivariable logistic regression using case variables was then undertaken to generate prognostic models. Centre and treatment were excluded from the prognostic models because they were not generalizable to other populations and were potentially subject to bias from financial constraint and clinician preference respectively. Previously, reported illness-severity scores (CHAOS and Tokyo) were not included in the multivariable analyses. Candidate predictor variables were chosen as follows: associated with outcome in the univariate regression at $P < 0.1$; no evidence of collinearity (correlation coefficient <0.9); an event:variable ratio >5.[25] For a complete case analysis to be performed in the prognostic models, only variables with <5% missing data were included.[26] Alanine aminotransferase (ALT), bilirubin, urea and creatinine values were indexed against (divided by) each centre's upper reference interval to account for variations in reference ranges. All variables were simultaneously entered into the model to maximize the predictive ability of prognostic models. Model accuracy was determined using 2 x 2 classification tables. Model discrimination was determined by calculating area under the receiver-operating characteristic curve (AUROC). Model calibration was assessed by Hosmer–Lemeshow goodness-of-fit (model rejected if $P < 0.05$) and visual inspection of the contingency table. Model utility was assessed using Nagelkerke's R^2.

Results

Retrospective and Prospective Case Comparisons

Although our aim was to enrol 335 cases, the rate of case recruitment was slower than anticipated. To minimize time-dependent changes in case management, the registry was closed early, limiting the study period to five years. Data from 276 cases (215 prospective, 61 retrospective) were collected. Only three variables differed significantly between retrospective and prospective populations (Table 2), which were therefore considered sufficiently comparable for subsequent combined analyses.

Study Population Characteristics

All centres contributed cases, median 18 (29) per centre. There were 246 (89.1%) purebred dogs and 30 (10.9%) cross-breeds. Two breeds were particularly prevalent: springer spaniels (n = 46, 16.7%) and cocker spaniels (n = 46, 16.7%). Spayed females represented 47.4% (n = 131), entire females 13.8% (n = 38), castrated males 27.2% (n = 75) and entire males 11.6% (n = 32). Fourteen dogs (5.1%) had a foreign-travel history. Thirteen dogs (4.7%) were vaccinated within 30 days of presentation. Many cases (n = 121, 43.8%) received medication in the month before presentation, typically for clinical signs attributable to IMHA: antibiotics (n = 63, 22.8%), glucocorticoids (n = 46, 16.7%), non-steroidal anti-inflammatory drugs (n = 31, 11.2%) and immunosuppressive (n = 13, 4.7%). Therapies for chronic disorders including epilepsy, diabetes mellitus, or hypothyroidism were administered to 31 dogs (11.2%).

Clinicopathologic Data

Two hundred twenty dogs were in-saline agglutination positive (79.7%), 85/113 dogs (75.2%) with a Coombs test result were positive, 183/254 (72.0%) dogs had moderate-marked spherocytosis. Blood typing for DEA 1 was attempted in 212 dogs (76.8%) and established in 180 (65.2%): 95 (34.4%) were positive and 85 (30.8%) negative. The median ASA classification was 3 (2–3), the median CHAOS was 3 (2–4) and the median Tokyo score was 3 (2–3); 58.3% fulfilled 2/4 SIRS criteria (n = 161), 19.9% fulfilled 3/4 SIRS criteria (n = 55).

Treatment

Most dogs (n = 244, 88.4%) received primary immunosuppressive, 101 dogs (36.6%) received two first-line drugs and nine (3.3%) received three (Table 3). Second-line immunosuppressive were used in 156 dogs (56.5%), 46 dogs (16.6%) received two additional drugs, and seven (2.5%) received three. Second-line drugs were instituted on median day 3 (2–5) after admission. Antithrombotics were administered to 210 dogs (76.0%) including: aspirin (n = 182, 65.9%), low molecular-weight heparin (n = 31, 11.1%), clopidogrel (n = 23, 8.3%) and unfractionated heparin (n = 10, 3.6%). Multiple antithrombotics were administered to 32 dogs (11.6%). Packed red blood cells were administered to 205 dogs (74.3%) (125 received 1 unit, 53 received 2 and 26 received ≥3). Fresh whole blood was administered to 21 dogs (7.6%) (17 received 1 unit and four received 2). Haemoglobin-based oxygen carrying solution was administered to 32 dogs (11.6%) (18 received 1 unit, 10 received 2 and four received 3). Only five dogs received fresh frozen plasma. More than one product type was administered to 35 dogs (12.7%).

Survival

Two hundred and five (74.3%) dogs were discharged from the hospital, equivalent to 25.7% mortality at discharge.

Table 2. Descriptive statistics of clinicopathologic data for the retrospective, prospective and combined populations. Variables significantly different between the prospective and retrospective populations (P < 0.05 after adjustment for multiple comparisons) are indicated in bold typeface.

	Retrospective		Prospective		Comparison	Combined	
Variable	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**P-value**	**Median (IQR)**	**n**
Age	7 (5)	61	6 (4)	214	0.25	6 (4.1)	275
ASA	3 (1)	51	3 (1)	215	0.73	3 (1)	266
Heart rate	128 (34)	61	130 (39)	208	0.43	130 (33)	269
Resp. rate	36 (24)	55	36 (16)	182	0.06	36 (19)	237
Temperature	101.3 (1.5)	60	101.7 (1.6)	205	0.07	101.7 (1.6)	265
Albumin	2.8 (0.7)	59	2.9 (0.6)	209	0.86	2.9 (0.6)	268
Globulin	3.4 (1.2)	59	3.3 (1.0)	209	0.89	3.3 (1.1)	268
ALT	**65 (195)**	**59**	**44 (85)**	**209**	**0.03**	**45 (98)**	**268**
ALP	198 (274)	59	184 (229)	210	0.08	189 (246)	269
Bilirubin	27.4 (81.6)	58	16.0 (53.5)	207	0.09	17.1 (59)	265
Urea	8.1 (6.3)	59	7.5 (5.1)	210	0.63	7.6 (5.3)	269
Creatinine	65 (24)	59	66.5 (28)	206	0.44	66 (27)	265
PCV	14.5 (8.0)	61	14.0 (7.0)	215	0.35	14.0 (7.2)	276
Leukocytes	26.5 (20.9)	61	21.6 (21)	213	0.17	22.5 (21.4)	274
Neutrophils	19.3 (17.1)	61	16.0 (17.2)	214	0.15	16.9 (17.2)	275
Monocytes	2.4 (3.7)	61	1.8 (3.0)	214	0.08	2.0 (3.0)	275
Platelet count	200 (72)	57	186 (173)	213	0.86	187 (147)	270
Retics	160.0 (234)	43	129.5 (224)	137	0.15	137 (226)	180
CHAOS	3 (2)	57	3 (2)	215	0.05	3 (2)	272
Tokyo	3 (1)	57	3 (1)	215	0.31	3 (1)	272
Polychromasia	2 (1)	57	2 (2)	199	0.09	2 (1)	256
Anisocytosis	**3 (1)**	**38**	**2 (1)**	**204**	**<0.01**	**2 (1)**	**242**
Spherocytosis	2 (2)	58	2 (2)	196	0.57	2 (2)	254
Breed					Breed variation P=0.57		
Sex					Sex variation P>0.99		
Drug (1st)					1st immunosuppressive drug variation P=0.96		
Drug (2nd)					2nd immunosuppressive drug variation P=0.94		
Antithrombotics					Antithrombotic drug variation P=0.84		
	+/-	**n**	**+/-**	**n**	**P-value**	**+/-**	**n**
Agglutination	50/11	61	170/45	215	0.72	220/56	276
Icterus	**31/27**	**58**	**75/135**	**210**	**0.02**	**106/162**	**268**
Pigmenturia	15/16	31	57/103	160	0.23	72/119	191
Travel	0/61	61	14/201	215	0.05	14/262	276
Medication	19/42	61	98/115	213	0.04	117/157	274
Vaccination	2/59	61	11/204	215	0.74	13/263	276
Discharged	46/15	61	159/56	215	0.87	205/71	276
Alive at 30d	43/17	60	143/71	214	0.53	186/88	274

ASA, American Society of Anesthesiologists physical status classification; ALT, alanine transaminase activity; ALP, alkaline phosphatase activity; Retics, absolute reticulocyte count. P < 0.03 were considered significant at the P < 0.05 level after adjustment for multiple comparisons.

Of the 71 non-survivors, 56 (20.3%) were euthanized and 15 (5.4%) died. Sixteen dogs (5.8%) were discharged but subsequently were euthanized or died and two dogs were lost to follow-up, such that 186 (67.4%) dogs were alive at 30 days after admission, equivalent to 30-day mortality of 32.6%. Twelve dogs underwent necropsy and no underlying diseases were identified.

Table 3. Summary of immunosuppressive and antithrombotic therapies administered to the study population.

First-line drug	n	% total pop.	Median (min–max) dose	Mode frequency
Dexamethasone	121	43.8	0.3 mg/kg (0.15–2)	Q24h
Prednisolone	121	43.7	1.3 mg/kg (0.5–2.2)	Q12 h
Azathioprine	78	28.2	2 mg/kg (1.23–2.5)	Q24 h
Cyclosporine	30	10.9	5 mg/kg (3–7)	Q24 h
hIVIG	5	1.8	0.5 g/kg (0.3–0.6)	Q24 h
MMF	4	1.4	15 mg/kg (13–15)	Q12 h
Second-line drug	**n**	**% total pop.**	**Median (min–max) dose**	**Mode frequency**
Prednisolone	78	28.2	1 mg/kg (0.65–2)	Q12 h
Azathioprine	39	14.1	2 mg/kg (1–4)	Q24 h
Cyclosporine	29	10.5	3.3 mg/kg (2–7)	Q12 h
MMF	10	3.6	10 mg/kg (5–20)	Q12 h
hIVIG	6	2.2	0.5 g/kg (0.2–0.5)	Q24 h
Cytarabine	3	1.1	50 mg/m^2 (50–100)	Q12 h
Dexamethasone	1	0.4	0.3 mg/kg	Q24 h
Cyclophosphamide	1	0.4	5 mg/kg	Q12 h
Antithrombotic	**n**	**% total pop.**	**Median (min–max) dose**	**Mode frequency**
Aspirin	182	65.9	0.5 mg/kg (0.25–5)	Q24 h
Dalteparin	31	11.2	150 IU/kg (100–250)	Q8 h
Clopidogrel	23	8.3	2 mg/kg (0.25–4.5)	Q24 h
UFH	10	3.6	200 IU/kg (75–200)	Q8 h

hIVIG, human immunoglobulin g; MMF, mycophenolate mofetil; UFH, unfractionated heparin.

Predictive Value of CHAOS and Tokyo

In univariate analyses CHAOS, when dichotomized as <3 or ≥3, was associated with death in hospital and death within 30 days of admission. Tokyo score, when dichotomized as <3 or ≥3, was not associated with any of the three outcome measures (Table 4). ROC curve data for CHAOS, Tokyo and the prognostic score from the multivariable models are reported in Table 5. The AUROC point estimate and 95% confidence intervals for CHAOS scores were higher than those for Tokyo scores. The 95% confidence intervals for Tokyo scores all included 0.5, suggesting it was little better than chance at predicting outcome in this population.

Outcome Modelling

In univariate analyses, eight candidate variables were associated with both survival at discharge and survival at 30 days (Table 4). There was no association between centre and death at discharge or at 30 days. Two variables were excluded for having >5% missing cases. Centre and the CHAOS and Tokyo scores were deliberately excluded from multivariable modelling. For survival prediction multivariable analyses, six variables were entered into the final model: SIRS, ASA classification, ALT, bilirubin, urea and creatinine. For prediction of outcome at discharge, this model was accurate in 82% of cases. In multivariate analysis, three variables were independently predictive of death in hospital: ASA classification, bilirubin and urea. Three variables were independently predictive of death by day 30: ASA classification, bilirubin and creatinine (Table 6).

Discussion

This multicentre study provides an overview of case characteristics, management and outcome for 276 dogs with primary IMHA treated at referral centres in the British Isles between 2008 and 2012. Despite intensive management with immunosuppressive, blood products and

Table 4. Results of univariate analyses for association with death at discharge and death within 30 days of admission. Variables significantly associated with the outcome variable (P < 0.1) are indicated in bold typeface.

Variable	Dead at discharge				Dead at 30 days			
	P value	OR	95% CI of OR		P value	OR	95% CI of OR	
			Lower	Upper			Lower	Upper
CHAOS score ≥3	**<0.01**	**4.162**	**2.178**	**7.951**	**<0.01**	**3.560**	**1.998**	**6.341**
Tokyo score	0.99	1.004	0.577	1.749	0.22	1.391	0.818	2.365
SIRS ≥3/4 criteria	**0.03**	**2.040**	**1.091**	**3.816**	**0.04**	**1.917**	**1.044**	**3.521**
ASA ≥3	**<0.01**	**3.205**	**1.541**	**6.665**	**<0.01**	**2.663**	**1.411**	**5.027**
Respiratory rate	0.20	1.010	0.995	1.026	0.35	1.007	0.992	1.023
Pigmenturia	**<0.01**	**2.536**	**1.330**	**4.834**	**0.01**	**2.184**	**1.186**	**4.024**
Autoagglutination	0.97	0.985	0.465	2.089	0.98	0.991	0.484	2.029
ALT (indexed)	**0.05**	**1.040**	**1.001**	**1.081**	**0.06**	**1.039**	**0.999**	**1.081**
Bilirubin (indexed)	**<0.01**	**1.013**	**1.005**	**1.020**	**<0.01**	**1.011**	**1.004**	**1.018**
Urea (indexed)	**<0.01**	**3.135**	**1.955**	**5.029**	**<0.01**	**2.587**	**1.666**	**4.018**
Creatinine (indexed)	0.06	2.356	0.956	5.804	<0.01	9.892	2.677	36.548

CHAOS, canine haemolytic anaemia objective score; SIRS, systemic inflammatory response syndrome; Abbreviations, ASA, American Society of Anesthesiologists physical status classification; ALT, alanine transaminase activity; OR, odds ratio.

Table 5. Comparison of the abilities of the Study model, CHAOS and Tokyo scores to predict death at discharge and day 30. Receiver operating characteristic curve results for the Study model, CHAOS and Tokyo scores.

Dead at day 30					
Score	**AUROC**	**SE**	**Asymptotic significance**	**Asymptotic 95% CI**	
				Lower	**Upper**
Study model	0.775	0.035	<0.001	0.706	0.844
CHAOS	0.688	0.036	<0.001	0.618	0.758
Tokyo	0.519	0.040	0.641	0.440	0.598

Dead at discharge					
Score	**AUROC**	**SE**	**Asymptotic significance**	**Asymptotic 95% CI**	
				Lower	**Upper**
Study model	0.729	0.036	<0.001	0.657	0.800
CHAOS	0.688	0.034	<0.001	0.622	0.755
Tokyo	0.542	0.037	0.261	0.470	0.615

AUROC, area under the receiver operating characteristic curve; SE, standard error; 95% CI, confidence intervals for odds ratio.

antithrombotics, the 30-day mortality rate was 32.6%. This figure is comparable to previous studies,[4,7,27,28] perhaps suggesting our ability to treat IMHA has not improved in recent years. Illness-severity scores might help identify dogs that might benefit from treatment intensification. This study evaluated the association of two IMHA specific illness severity scores with outcome in our population. Of these, CHAOS ≥3 was associated with increased odds of death and in particular, a high CHAOS was associated with a risk of death during hospitalization. Tokyo score was not useful for outcome prediction in our study. We also found that ASA classification ≥3 was also associated with death, suggesting the subjective assessment of experienced clinicians can be a reasonable gauge of illness severity in IMHA. As can be seen from the AUROC values (Table 5), the final multivariate model allows outcome to be predicted more accurately than previously reported scoring systems. This is not unexpected, since a model generated from our data should describe our population better than those derived from other populations, and independent evaluation of our model in an unrelated population should be undertaken to ensure its validity. It is noteworthy

Table 6. Summary of multivariable logistic regression models. Variables significantly associated with the outcome variable (P < 0.05) are indicated in bold typeface.

Summary of independent predictive variables for death at discharge						
Variable	**Coefficient (B)**	**SE**	**P-value**	**Odds ratio (OR)**	**95% CI for OR**	
					Lower	**Upper**
SIRS ≥3/4 criteria	0.233	0.410	0.57	1.263	0.565	2.823
ASA ≥3	**0.997**	**0.475**	**0.04**	**2.709**	**1.068**	**6.870**
ALT (indexed)	0.028	0.019	0.15	1.028	0.990	1.067
Bilirubin (indexed)	**0.010**	**0.004**	**<0.01**	**1.010**	**1.003**	**1.017**
Urea (indexed)	**1.114**	**0.331**	**<0.01**	**3.046**	**1.592**	**5.830**
Creatinine (indexed)	-0.639	0.588	0.28	0.528	0.167	1.670
Constant	3.333	0.507				

AUROC	0.775 (95% CI 0.706–0.844)
Hosmer–Lemeshow P value	0.31
Nagelkerke's R^2	0.304

Summary of independent predictive variables for death at 30-days						
Variable	**Coefficient (B)**	**SE**	**P-value**	**Odds ratio (OR)**	**95% CI for OR**	
					Lower	**Upper**
SIRS ≥3/4 criteria	0.201	0.384	0.60	1.222	0.576	2.593
ASA ≥3	**0.776**	**0.386**	**0.04**	**2.173**	**1.020**	**4.629**
ALT (indexed)	0.025	0.018	0.17	1.026	0.989	1.064
Bilirubin (indexed)	**0.009**	**0.003**	**0.01**	**1.009**	**1.002**	**1.016**
Urea (indexed)	0.440	0.274	0.11	1.552	0.907	2.655
Creatinine (indexed)	**2.197**	**0.902**	**0.02**	**8.996**	**1.537**	**52.672**
Constant	-3.449	0.579				

AUROC	0.729 (95% CI 0.657–0.800)
Hosmer–Lemeshow P value	0.89
Nagelkerke's R^2	0.261

SE, standard error; 95% CI, confidence intervals for odds ratio; AUROC, area under the receiver operating characteristic probability curve; OR, odds ratio.

however that of the two previously developed scores, the AUROC point estimate and 95% confidence intervals for CHAOS were good, while the 95% confidence intervals for Tokyo included 0.5, suggesting it was little better than chance at predicting outcome in our population.

We used logistic regression analysis to assess the association of individual clinical and clinicopathologic variables with outcome. Individual variables with significant associations with outcome were combined into multivariable models and the accuracy of these models evaluated using 2 x 2 classification tables that identify how many dogs were correctly classified as dead or alive. The AUROC values were higher for the final multivariable model than for any of the individual variables alone (data not shown). The final six-variable model for prediction of outcome at discharge was accurate in 82% cases. Although this suggests the model was highly accurate, it should be noted that assuming every dog was discharged alive would have been correct in 74% of cases. The R^2 values suggest that the six variables (SIRS, ASA classification, ALT, bilirubin, urea and creatinine) included in our death in hospital model represent only a minority of the factors determining outcome. In linear regression, a model containing all the variables needed to explain outcome has $R^2 = 1$. While R^2 values in logistic regression are pseudo-R^2, their interpretation is similar. For example, our six-variable model predicting death during hospitalization had $R^2 = 0.304$. This indicates that most of the factors that influence the likelihood of death during hospitalization were not included in our model. These unquantified variables might be unidentified or unmeasured case factors, the effects of treatment and complications including thrombosis.

Seventy-two dogs died before discharge, while a further 16 did not survive to 30 days. Three variables were predictive of outcome at both times, suggesting some consistency between the causes of death during hospitalization and at 30 days. There was one difference in the prediction models between outcomes at discharge versus 30 days: urea was independently predictive of outcome at discharge but not at 30 days, while creatinine was not independently predictive of outcome at discharge but was at 30 days. The cause of these differences is unclear. The association between creatinine concentration and outcome at 30 days might suggest that end-organ dysfunction associated with hypoxemia, nephrotoxicity from drug administration, or haemoglobinemia affects medium- term outcome. Acute kidney injury is associated with non-survival in critically-ill dogs,[29] and even small deteriorations in kidney function can affect outcome.[30]

Our intention was to test our hypotheses by enrolling 335 dogs; however, we were only successful in recruiting 276 dogs within a 5-year period. Although this might have reduced our ability to identify reliable prognostic markers, post hoc power calculations suggested we were powered to detect a 2.02-fold change in case fatality, which was almost exactly our goal. This study combined retrospective and prospectively collected data into 1 dataset, to maximize the data available for analysis, while minimizing the time taken for data collection. We compared demographics, clinicopathologic data and treatment data for these populations prior to combining them. Three statistically significant differences were identified between these two populations (ALT activity, icterus frequency and anisocytosis severity), but the clinical relevance of these differences is debatable. The absence of a difference in outcomes between the retrospective and the prospective groups supports this assertion. We cannot exclude the possibility that other differences might have existed that would affect the validity of our approach, but all data from the two parts of the study were collected from the same group of centres, which should improve the homogeneity of the data. Overall, we feel that the two populations were not clinically different sufficient that this would affect the results of our evaluation of associations using data from the combined population.

Our analyses attempted to account for differences in the reference intervals between centres. After initial screening, it was determined that some variables should be indexed to the institution's reference intervals. Since this was not performed for all variables, it is possible we might have overlooked some significant associations between non-indexed variables and outcome. However all of the variables in the final models were indexed, which maximizes their generalizability. We also considered that centre might have had an effect on outcome either through distinct case demographics or institutional differences in case management or treatment availability. To address this, we evaluated the association of centre with outcomes in univariate analyses. We found no significant effect of centre on outcome in these analyses. Centre was deliberately excluded from the multivariable analyses to maximize generalizability, but regardless centre would not have been included in our multivariable models on the basis of a lack of association in the univariate analyses. This argues that if distinct treatment strategies employed by different institutions significantly influenced outcome then an effect of centre on outcome might have been found, but was not. The effects of treatment on outcome were not evaluated by this study directly, but warrant investigation in prospective interventional trials.

Although tailored diagnostic evaluation was performed in all of the cases to identify an underlying mechanism for their IMHA, this was not exhaustive and thus we cannot exclude the possibility that some of these dogs had an unidentified primary cause. For instance, many dogs underwent PCR screening for vector-borne pathogens, but few underwent more complete screening as has been recently recommended.[31] The potential effect of this is difficult to quantitate, since unidentified primary causes of IMHA might be expected to worsen the prognosis by perpetuating the generation of autoantibodies. Other limitations inherent in this study are biases induced by financial limitations and euthanasia. Assessing dogs managed only at referral centres might have reduced these effects by reducing the number of dogs euthanized for financial limitations. Most deaths in this study were due to euthanasia, however, with the inherent potential for confounding by euthanasia for reasons other than illness-severity or lack of response to treatment that is difficult to codify or exclude.

Conclusion

This large multicentre cohort study provides insight into the current management and outcome of dogs with IMHA treated in the British Isles. Two previously published illness-severity scores (CHAOS and Tokyo) were

prospectively evaluated for their ability to predict outcome in a population separate from that used to generate the score. Of these two, only CHAOS was predictive of outcome in our population. Using our large dataset, we identified that markers of kidney function, bilirubin concentration, and ASA classification are independently associated with outcome in dogs with IMHA; a multivariate model combining illness severity scores and clinicopathologic data correctly predicted outcome at discharge in 82% cases. The ability of the factors identified here to predict outcome can now be evaluated in other populations, ideally before use as a means to stratify dogs for prospective interventional trials.

Footnotes

a Whelan MF, Rozanski EA, O'Toole TE, et al. Use of the canine haemolytic anaemia objective score (CHAOS) to predict survival in dogs with immune mediated haemolytic anaemia. J Vet Intern Med 2006; 20:714–715 [Abstract]

b http://wwwn.cdc.gov/epiinfo/html/ei6_downloads.htm

c https://www.surveymonkey.com

d Excel 2010, Microsoft

e SPSS 21, IBM

f Prism 5.0, GraphPad

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Conflict of Interest Declaration:
Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration:
Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Summary of diagnostic testing undertaken to exclude primary causes of IMHA in the study population.
http://goo.gl/Whl2vD

Data S1. Final database containing demographic, clinical, clinicopathologic, treatment and outcome data.
http://goo.gl/fc6GRq

Data S2. A printable version of the custom online survey form used to facilitate data entry.
http://goo.gl/8Dsl8q

Reprint paper*

Health screening to identify opportunities to improve preventive medicine in cats and dogs

Marianne Diez[1], Philippe Picavet, Rebecca Ricci, Marjorie Dequenne, Marcel Renard, Alexandre Bongartz and Frédéric Farnir

ABSTRACT

Objectives: To describe the results of a prevention campaign in terms of participation and pet health status and to identify opportunities to improve preventive medicine in cats and dogs.

Methods: An awareness campaign was designed to highlight the role of veterinarians and emphasise the benefits of a veterinary visit. Owners were invited to make an appointment for a free pet health check in a voluntarily participating veterinary clinic. Observations recorded by the veterinarians were entered in a database and subsequently analysed using simple descriptive statistics.

Results: A total of 5305 completed health check forms were analysed. The percentages of overweight and obese dogs and cats were 34 and 36%, respectively; this was the most common finding, followed by dental calculus (31% in dogs, 21% in cats). In total 67% of cats did not undergo flea control and 59% were not vaccinated.

Clinical Significance: Opportunities for increased quality of care are numerous given the high percentage of intact, unvaccinated or non-permanently identified pets and the low level of worm and flea control. Animal health should benefit from preventive measures, and improved management can be undertaken after early detection of diseases.

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Introduction

In Belgium, the percentage of pets receiving veterinary care are estimated at 25 to 30% for cats and 40 to 55% for dogs (Degallaix 2014). Pets are rarely insured and there is no national database for medical care and frequency of disease or accidents. Many pets do not have basic preventive health care, as reported in the UK in the PDSA (2013). Because of the lack of routine check-ups, chronic diseases affecting old pets may not be detected early. In contrast, preventive medicine is currently developing and implementing specific programs, for example vaccination, nutrition and geriatric health care are being actively recommended (World Small Animal Veterinary Association – WSAVA 2010, Freeman et al. 2011, Fortney 2012).

In this context, a major awareness campaign was designed in 2011 in the French-speaking part of Belgium (Brussels and south of Belgium).

The key principle was to offer owners the opportunity for their pet(s) to be given a physical health check free of charge. After an evaluation of preventive health care (vaccination, flea and worm control, body condition score and quality of diet and health status), clinical recommendations were given by the veterinarian.

Several objectives were taken into account in the development, set-up and design of the campaign: (1) to promote the roles of the veterinarian and regular visits for a complete

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health check-up, preventive medicine and to stimulate health care follow-up; (2) to analyse the data of the animals participating in the campaign and to obtain figures on preventive medicine and health status from a large pet population; and (3) to communicate the results to veterinarians and owners.
This report presents the main results of the campaign in terms of participation, pet health status and opportunities for the improvement of preventive medicine in dogs and cats.

Materials and methods

Design of the prevention awareness campaign

After preparation and agreement on the mechanism and design, the French-speaking Small Animal Veterinary Association of Belgium (SAVAB) informed all veterinary practices of the campaign's mechanism and objectives by post, e-mail and with a dedicated website (http://www.saisondelaprevention.be) providing the participation form, registration rules, and practical support in running the campaign. Veterinarians were invited to register on a voluntary basis and be listed as participants. By doing so, they agreed to allow prior and newly registered owners to present their pet(s) for a free health status check-up during the month of February 2011.

Practical support consisted of materials that explained the campaign to participating veterinarians, an invitation letter, leaflets, a frequently asked questions document, a waiting room poster, written information for the owners, and the health check form and a pet health guide to be distributed after the free health check. Another website, dedicated to owner registration, allowed them to provide their written consent and stated that no treatment or vaccination would be provided for free.

The health check form contained three parts (Table 1). The first part recorded owner details: name and address, animal description and questions about diet, housing, travel, vaccination, means of identification, veterinary visits, and parasite prevention including deworming status. The second part contained the data collected from physical examination, including bodyweight in kg (BW), body condition score (BCS) on a 5-point scale, and by system: Items 1 to 10 listed on the health check form. After physical examination, the veterinarian was also required to assess vaccination and deworming status, and the adequacy of the diet. For each item/system, the veterinarian selected "normal" or "abnormal" and added remarks. It must be noted that in animals receiving veterinary care, veterinarians completed the form using terms such as "previously identified condition" or "treated for disease."
The third part of the health check form presented the follow-up care recommendations based on the abnormalities noted and also recorded any follow-up appointment made (e.g. blood or urine analysis, X-ray, therapy or surgery). For each animal presented, more than one disease could be recorded. Animals presenting without any obvious disease and with a BCS of 3/5 were considered healthy. Veterinarians were asked to be as precise and thorough as possible when completing the form. Owners received a written summary of the problems and recommendations.

Before the campaign, the health check form was tested at the veterinary faculty of Liège for 2 weeks. Thirty completed health check forms were obtained from four internal medicine residents and minor changes were made to specify the type of housing and the usual diet.

In order to participate, pet owners were asked to register themselves and their pets through the online website or via the call centre and to confirm their understanding of both the definition of the free health check and the participation rules. They had to print the health check form (Table 1) and take it to a participating veterinarian, to make an appointment, and to have the form completed by the veterinarian during the check. The forms returned by the veterinarians would then be collected by the SAVAB, processed and analysed by the Faculty of Veterinary Medicine, and a donation would be made (1 for each form collected) to the Guide Dogs for The Blind Association (www.scaledogs.be).

In order to inform pet owners of the prevention campaign, a broad media campaign was developed and launched from January 15 to February 20, 2011. The campaign was open to all dogs and cats whether they had visited a veterinarian previously or not. Participating owners and veterinarians were informed that the data resulting from the health check would be used for epidemiological analyses to study the population (Table 1).

Data collection

The returned forms were encoded in an Access® (Microsoft) database by two veterinary students. These students (fourth year of the curriculum) were trained for 3 hours and coached by two senior veterinarians (first and co-author of this paper). They were randomly selected to process half

Table 1. Content of the health check form completed by the veterinarian

Season of Prevention 2011 – Health check form

Date:

Owner
Name:
Surname:
City: City code:
Email:

Identification
☐ Microchip
☐ Tattoo
☐ None

Visit to a vet (in the last 12 months)
☐ Yes
☐ No
☐ Animal never visited a vet

Animal
Name:
Species: ☐ Dog ☐ Cat
Breed:
Birth date: Age (years):

During the last 12 months
Travel abroad: ☐ Yes ☐ No If Yes: country:
Deworming: ☐ Yes ☐ No If Yes: frequency:
External anti-parasites: ☐ Fleas ☐ Ticks ☐ Others Frequency:

Environment
☐ City ☐ Country
☐ Apartment ☐ Outdoor access

Gender
☐ F ☐ SF ☐ M ☐ CM

Diet
☐ Home-made diet
☐ Commercial diet

Type:
☐ dry ☐ wet
☐ Mixed diet (home-made + commercial)

Bodyweight (kg):

Body condition score (BCS)
☐ 1 (very thin)
☐ 2 (thin)
☐ 3 (normal)
☐ 4 (overweight)
☐ 5 (obese)

Clinical examination	Normal	Observed problems	Remarks
1. BW /BCS			
2. Skin			
3. Mouth – Teeth			
4. Ears & hearing			
5. Cardiac system			
6. Respiratory system			
7. Gastro-intestinal system			
8. Urinary system			
9. Genital system			
10. Locomotor system			
11. Vaccination status (last 12 months)			
12. Deworming status			
13. Diet adapted to health/life/age			
14. Follow-up Recommendation			

An appointment has been taken with the owner at this date: / /

Stamp, date and signature of the vet:

To be sent to SAVAB before the end of March
For each completed form, 1 euro will be given to the association SCALE dogs, to support the training of guide dogs for the blind.
Data of this form will be collected and analysed in collaboration with the Companion animal Nutrition Unit of the Veterinary Faculty of the University of Liège.

of the forms and allowed to request guidance from senior veterinarians for doubtful records to ensure accurate data entry.

All data reported on the forms (Table 1) were included in the database. Breed data were entered using a menu list with the possibility of adding new breeds. Any breed combination was coded as a mixed breed. Diagnostic categories included the Items 1 to 10 used in the form (or location code) and diagnostic codes. The list of diagnostic codes was dynamic, and the number of terms and synonyms grew with participant use. This permitted all levels of definition of a sign or a diagnosis to be collected, from a vague problem (e.g. polyuria) to a specific diagnosis (e.g. known renal disease). Terms and codes were matched to the Systematized Nomenclature for Medicine and Veterinary Medicine to facilitate analysis and future comparisons (College of American Pathologists 2002).

Statistical methods

The Access® database was used to generate prevalence estimates. The prevalence of the various disorders was calculated by dividing the number of cats or dogs for which the specific diagnostic code had been recorded at least once during the study by the total number of cats and dogs presented during the same period. Confidence intervals, with confidence levels of 95%, were estimated using an exact binomial method in a Microsoft® Excel spreadsheet (Clopper & Pearson 1934). No correction for multiple testing was performed, which reinforces the need to consider the results reported as significant with some caution. Associations between age classes and various disorders were tested using Chi-square tests on the corresponding contingency tables. A value of <0.05 was considered significant.

RESULTS

Practice and owner participation

In total, 470 veterinary practices (791 veterinarians, 60% of the veterinarians registered as companion animal practitioners in the same area) registered to participate in the campaign. Among them, 350 veterinary practices returned at least one completed form. A total of 13,287 pet owners registered a total of 17,938 pets (57% dogs, 43% cats). A total of 5305 (56% dogs, 44% cats) completed health check forms were returned.

Population description

Age distributions for cats (n=2260) and dogs (n=2929) are presented in Fig 1. Because of the observed asymmetry in the age distributions, medians were computed and values of 5.0 (IQR – 25th percentile subtracted from the 75th percentile – 6.7) and 4.5 (IQR 7.2) years were obtained for dogs and cats, respectively. The age of 12% of the dogs and 17% of the cats were below one year, while 41% of the dogs and 36% of the cats were above seven years of age. Medians of BW for the dog and cat populations were 12 (IQR 16) and 4 (IQR 4) kg, respectively. Table 2 presents data on gender and breed. Information on diet was provided for 2796 dogs and 2319 cats. For most cats (83%) and dogs (65%), the major diet component was a commercial food; 16% of cats and 30% of dogs were fed mixed diets (commercial

FIG 1. Age (year) distribution (%) for 2260 cats (■) and 2929 dogs (■) examined at private practices during the prevention campaign

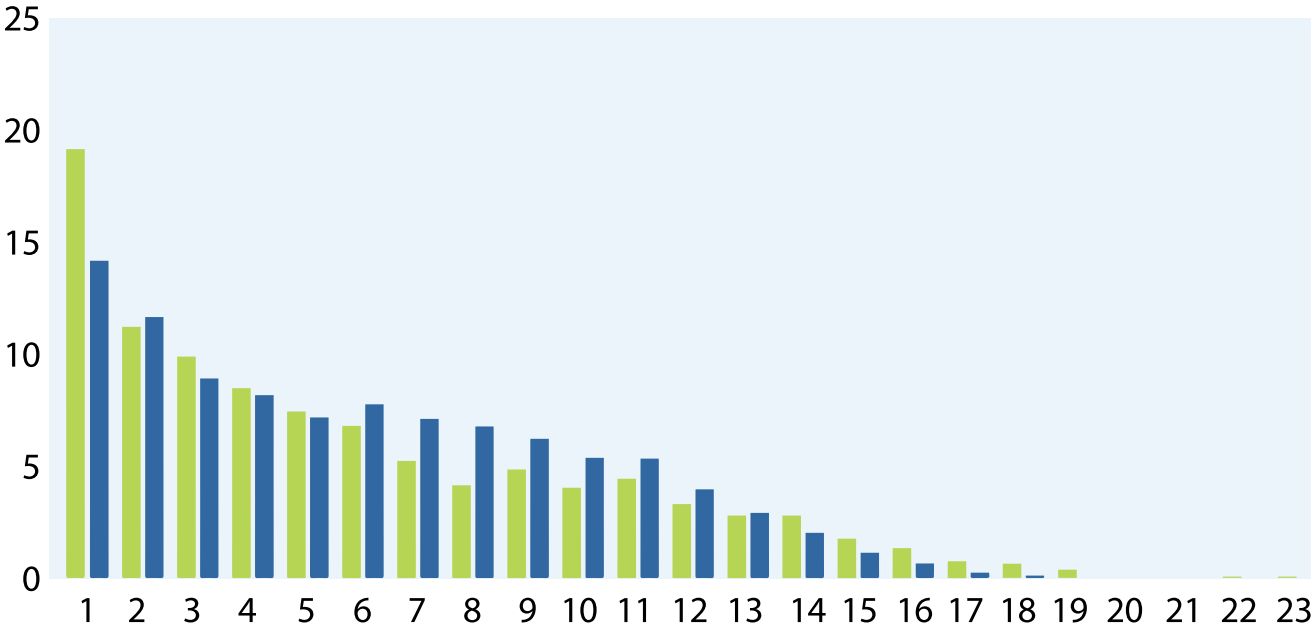

Table 2. Summary of dog and cat characteristics [gender (%) and breed (%)]

	Dogs	Cats	
Gender (%)	**(n=2474)**	**(n=1974)**	
Intact males	36	11	
Neutered males	14	36	
Intact females	28	17	
Neutered females	22	36	
Breeds (%)	**(n=2888)**	**(n=2178)**	
Mixed breeds	23	18	
Pure breeds	77	82	
Bichon	7.8	*Domestic shorthair*	68
Yorkshire terrier	6.3	*Persian*	2.7
Labrador retriever	4.2	*Siamese*	2.6
Golden retriever	3.7	*British shorthair*	2.1
Jack Russel terrier	3.7	*Burmese*	1.7
Shih-tzu	3.5	*Other breeds*	*4.9*
Chihuahua	3.3		
Border collie	2.5		
Cocker spaniel	2.5		
German shepherd	2.3		
French and English bulldogs	2.1		
Other breeds	*35.1*		

and homemade). Few cats (0.8%) and dogs (5%) were fed homemade diets only. Animals were determined to be overweight or obese when the BCS were 4 and 5, respectively (Table 1). A majority of dogs (62%) and cats (58%) presented with a normal BCS of 3/5, 28% of them with a BCS of 4/5 and thus 4.5% of dogs and 7.9% of cats were considered obese with a BCS of 5/5. Only 5% of the dogs and 6% of the cats were considered thin or very thin.

Preventive medicine

According to the forms completed by the veterinarians, based on the declarations of the owners, 66% of dogs and 43% of cats had been seen by a veterinarian during the last year; 7% of dogs and cats never had a visit to a veterinarian and the remainder (27% of dogs and 50% of cats) had not been seen by a veterinarian during the last year. Data are presented for dogs and cats in Table 3.

Because of the high percentage of unidentified or unvaccinated animals, the data were studied separately based on whether the animals had received veterinary care (at least one visit to the veterinarian during the previous 12 months) or not. The percentages of animals that had received veterinary care, without being vaccinated, identified or dewormed, are presented in Table 3.

Table 3. Data for preventive medicine in dogs (n=2986) and cats (n=2319)

Preventive medicine - DOG	Number of answers	Problems*			Recommendation†	Treatment or appointment‡
		All dogs	No Vet care	Vet care		
Body weight - BCS >3	2525	847 (34%)	287 (12%)	560 (22%)	213 (25%)	0
No microchip	2830	388 (14%)	207 (7%)	181 (7%)	51 (13%)	0
Not vaccinated	2972	964 (32%)	631 (21%)	333 (11%)	431 (45%)	25 (6%)
No prevention against fleas	2479	1166 (47%)	417 (17%)	749 (30%)	57 (5%)	0
No prevention against internal parasites	2770	846 (31%)	449 (16%)	397 (15%)	319 (38%)	0

Preventive medicine - CAT	Number of answers	Problems*			Recommendation†	Treatment or appointment‡
		All dogs	No Vet care	Vet care		
Body weight - BCS >3	1877	682 (36%)	335 (18%)	347 (18%)	159 (23%)	0
No microchip	2115	1824 (86%)	1012 (48%)	812 (38%)	17 (0.9%)	0
Not vaccinated	2309	1351 (59%)	980 (43%)	371 (16%)	417 (31%)	11 (0.3%)
No prevention against fleas	1845	1239 (67%)	690 (37%)	549 (30%)	146 (12%)	0
No prevention against internal parasites	2170	979 (45%)	695 (32%)	284 (13%)	321 (33%)	0

Vet care: animals presented at a veterinary practice during the 12 months before the study
No Vet care: animals not presented at a veterinary practice during the 12 months before the study or animals never presented at a veterinary practice
* Percentage of animals presenting with a problem
† Percentage of animals receiving the recommendation linked to the identified problem
‡ Percentage of animals receiving an appointment linked to the identified problem and the recommendation

Table 4. The most common disorders reported for 2986 dogs examined at private veterinary practices during the prevention campaign and the associations between age classes and disorder prevalence (P)

Disorder	Prevalence % total	95% CI	Prevalence % by age classes			Probability
			<24 months	Two to six years	Above Six years	
Body weight- BCS>3/5	33.5	31.7 to 35.7	9.8	38.5	51.7	<0.001
Dental calculus	31.1	29.5 to 32.8	7.2	29.4	49.2	<0.001
Otitis externa	14.0	12.8 to 15.3	13.1	13.6	15.0	0.459
Mammary tumours*	11.2	8.9 to 13.6	2.0	5.8	26.6	<0.001
Cataract	9.5	8.4 to 10.5	0.4	1.2	24.4	<0.001
Heart disease	6.4	5.5 to 7.3	1.2	2.2	14.4	<0.001
Osteoarthritis	5.4	4.5 to 6.2	0.5	1.2	13.1	<0.001
Lameness	5.4	4.5 to 6.2	3.9	5.0	6.9	0.013
Dry hair and dandruff	4.1	3.4 to 4.8	2.7	4.1	5.1	0.040
Gingivitis	3.4	2.8 to 4.0	0.3	2.3	6.8	<0.001
Respiratory tract diseases	3.2	2.6 to 3.9	1.7	2.4	5.1	<0.001
Lump	3.0	2.3 to 3.6	0.7	1.9	5.7	<0.001
Flea infestation	2.8	2.9 to 4.4	3.2	2.5	2.9	0.665
Moist dermatitis	2.8	2.2 to 3.5	0.5	3.1	4.1	<0.001
Atopic/allergic dermatitis	2.7	2.1 to 3.3	1.3	3.1	3.1	0.031
Conjunctivitis	2.6	2.0 to 3.2	2.9	1.5	3.7	0.004
Dermatitis	2.3	1.8 to 2.9	0.9	3.1	2.5	0.008
Patellar luxation	2.2	1.7 to 2.8	1.6	2.4	2.5	0.391
Anxiety	2.0	1.5 to 2.6	2.7	1.7	2.0	0.309
Disk disease	2.0	1.5 to 2.5	0.4	1.0	4.3	<0.001

*Incidence of mammary tumours was calculated in entire females >12 months

Disease prevalence

In total 27% of the dogs (8% not receiving veterinary care and 19% receiving veterinary care) and 31% of the cats (16% not receiving veterinary care and 15% receiving veterinary care) were considered healthy (having a BCS of 3/5 and no diagnostic codes). Tables 4 and 5 present summary statistics for the main diseases. Many reported disorders were common to both dogs and cats (e.g. flea infestation or conjunctivitis) and age-related. Overweight condition and obesity were the most commonly reported disorders for both species (Tables 3–5). In the dog, the frequency of mammary tumours was higher (P<0.001) in entire (11.2%) than in neutered females (1.3%).

Recommendations and follow-up

During the health checks, veterinarians wrote 2957 and 2467 recommendations for the dogs and the cats, respectively. The number of recommendations ranged from 0 (40%) to 5 in cats and from 0 (42%) to 7 in dogs; 29% of cats and 31% of dogs received one recommendation; the remaining animals (27% of dogs and 31% of cats) received more than one recommendation. The main recommendations for the dogs were the following: vaccination (18% of all recommendations), changing the diet (17%), deworming (17%), further examination in internal medicine (11%), dental care (10%) and implementing a weight loss programme (7%). For the cats, the main recommendations were: deworming (20% of all recommendations), vaccination (20%), changing the diet (16%), flea control and further examination in internal medicine (9% each), dental care (8%) and neutering (6%). Veterinarian recommendations linked to known problems are presented in Table 3. Although pet identification is compulsory for dogs in Belgium, the recommendation was made for 1.7% of dogs and 0.9% of cats. Finally, 16% of dogs and 15% of cats were given an appointment for a follow-up visit.

Discussion

The data presented in this study cannot be compared to any other study performed in Belgium as it is the first time that the campaign has been organised and the results recorded. While such information can sometimes be gained from questionnaire surveys, greater precision requires the

Table 5. The most common disorders reported for 2319 cats examined at private veterinary practices during the prevention campaign and the associations between age classes and disorder prevalence (P)

Disorder	Prevalence % total	95% CI	Prevalence % by age classes			Probability
			<24 months	Two to six years	Above Six years	
Body weight-BCS > 3/5	36.3	34.2 to 38.5	15.2	42.8	41.9	<0.001
Dental calculus	21.4	19.7 to 23.0	4.0	17.6	41.5	<0.001
Gingivitis	11.3	10.0 to 12.5	6.2	10.0	17.4	<0.001
Otodectes spp infestation	8.0	6.9 to 9.1	12.1	5.3	7.2	<0.001
Flea infestation	7.8	6.7 to 8.9	7.9	7.4	8.1	0.849
Otitis externa	5.5	4.5 to 6.4	4.0	6.1	6.0	0.152
Dry hair and dandruff	4.4	3.6 to 5.2	1.7	4.3	7.0	<0.001
Respiratory tract infection	3.9	2.9 to 4.4	3.9	4.0	3.7	0.946
Conjunctivitis	3.8	2.8 to 4.3	4.5	2.9	4.1	0.212
Teeth - broken or lack of-	3.3	2.6 to 4.0	1.6	2.3	6.0	<0.001
Hair loss	2.9	2.2 to 3.6	1.7	3.6	3.3	0.077
Feline miliary dermatitis	2.8	2.1 to 3.5	1.3	3.1	3.8	0.011
Atopic/allergic dermatitis	2.3	1.7 to 2.9	1.2	2.2	3.6	0.009
Heart disease	2.3	1.7 to 2.9	0.6	1.2	5.3	<0.001
Renal disease	2.0	1.5 to 2.6	0.1	0.7	5.2	<0.001
Osteoarthritis	1.6	1.1 to 2.2	0.1	0.5	4.4	<0.001
Dermatitis	1.5	1.0 to 2.0	0.7	2.0	1.6	0.124
Cataract	1.5	1.0 to 2.0	0.0	0.2	4.2	<0.001
Stomatitis	1.4	0.9 to 1.9	1.3	1.5	1.3	0.934
Feline urologic syndrome	1.2	0.8 to 1.7	0.7	1.0	1.9	0.120

*Incidence of mammary tumours was calculated in entire females >12 months

direct assessment of the dog and cat population as made by the voluntarily participating veterinarians in this study. In the UK, the PDSA charity trust provides annual reports on the health and preventive care of pets in different areas of the country as an important tool that helps the veterinary profession understand and meet the needs of the owners and animals (PDSA 2013). One of the goals of the present study was to emphasise the importance of preventive care to owners and veterinary professionals; the data show this to be an important issue in veterinary medicine and also essential to public health (e.g. deworming in cats) (Macpherson 2013).

Pets "receiving veterinary care" were defined as those that had been seen by a veterinarian in the last year and in most practice management software programmes, these are also identified as "active patients" if presented during the last 13 months. During the free health check (as reported on the forms), 7% of owners declared that they had never been to a veterinarian. The accuracy of these data, and consequently the figures on the status of pets receiving veterinary care (presented at a veterinary practice during the last year) cannot be entirely verified however, and thus, must be considered with caution as perhaps being under- or overestimated.

One of the most interesting findings shows that a large proportion of pets receiving veterinary care received little preventive care. The proportion of unvaccinated animals – even against rabies which is compulsory – is high in both species. This can be partly explained by the design of the campaign, which aimed at stimulating the participation of owners who do not visit a veterinary practice regularly. However, the results are based on the health check forms and in most cases, recommendations might also be given orally.

The lack of preventive care was higher in the cat population than that in dogs. For example, 14% of the cats in the present study had a microchip; yet as many as 46% of cats in the UK had a microchip the same year (PDSA 2013). It appears that many veterinarians do not actively recommend microchipping, as shown by the low percentage (0.9%) of cat owners receiving such recommendation.

Neutering is generally considered as responsible pet ownership (RSPCA 2014); in the present study, 72% of cats were neutered when compared with only 36% of dogs. As of September 1, 2014, neutering and microchipping of all newborn cats (DSH and other breeds) is compulsory in Belgium, with derogations for professional breeders. The high percentage of entire bitches suffering from mammary tumours (26% of entire females older than six years) suggests that the role of neutering in young pet female dogs to reduce mammary cancer incidence should be re-considered, despite the limited published evidence that neutering protects against mammary neoplasia (Beauvais et al. 2012).

The discussion is limited to highly prevalent chronic diseases because the results do not reflect the usual work of a veterinary practice, given that the design of the campaign virtually excluded the participation of animals in acute conditions (e.g. gastrointestinal diseases or acute pain). The high percentage of overweight and obese dogs and cats, 34 and 36%, respectively, of the population studied, was not surprising. These conditions are common medical disorders in pets in the countries in which studies have been conducted (Lund et al. 1999, Colliard et al. 2009). In this study, it is also interesting to note that for this specific overweight indication, the correct recommendation of a weight loss plan including dietary management was given to only 25% of the affected dogs and 23% of the cats. Making an effective recommendation is nevertheless key to ensure the quality of care after assessments of nutritional status with BCS and BW (Wayner & Heinke 2006, AAHA 2011, Freeman et al. 2011).

The second key health issue identified was linked to oral health: dental calculus was common, and this was consistent with previous studies (Lund et al. 1999). Dental calculus has been associated with systemic disease (DeBowes 1998) and its key preventive and management principles are known (Logan et al. 2010). In the present study, most animals did not receive a recommendation in this regard. This gap observed between the diagnosis and the recommendation has also been well documented by another study (AAHA 2003). Awareness of this situation along with protocols and systematic health care team approaches within the practice may be considered for the improvement of compliance (Wayner 2010).

On the basis of physical examination, 27% of dogs and 31% of cats were considered healthy; however, it has been shown that apparently healthy middle-aged and old cats suffer from many diseases including high systolic blood pressure or crystalluria and that regular health checks, including further examinations, are beneficial (Verjans et al. 2011). A thorough clinical examination conducted at the time of routine vaccination also appeared to be an important element in maintaining animal health and welfare (Banyard 1998, WSAVA 2010). Screening elderly dogs also identified unrecognised and unreported health risk factors resulting in lifestyle modification and ongoing monitoring, as well as signs of age-related diseases. This results in diagnostic investigations, early diagnoses and surgical and medical interventions to improve quality of life (Davies 2012, Fortney 2012).

The data collection procedure adopted might raise some questions on the representativeness of the sample: voluntary participation of this kind is likely to introduce certain biases that might limit some of the conclusions drawn in the study. In summary, the results of this study suggest that there are numerous opportunities to improve preventive medicine and increase the quality of care in the pet population given the high percentage of intact, unvaccinated or unidentified animals and the low level of systematic preventive care against worms and fleas. At the same time, the most frequently reported problems can be managed by veterinarians, and preventive measures can be taken to avoid these in healthy pets through adequate communication and clear recommendations including application of WSAVA nutritional guidelines (Freeman et al. 2011).

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Conflict of interest

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