PAPER

Enteropathogens in pups from pet shops and breeding facilities

S. Dupont*,†, P. Butaye‡,§, E. Claerebout¶, S. Theuns¶, L. Duchateau||, I. Van de Maele* and S. Daminet*

*Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke B-9820, Belgium

†Veos N.V., Akkerstraat 4A, Zwevezele B-8750, Belgium

‡Coda-Cerva, Veterinary and Agrochemical Research Centre, Groeselenberg 99, Brussels B-1180, Belgium

§Department of Pathology, Bacteriology, and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke B-9820, Belgium

¶Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke B-9820, Belgium

||Department of Physiology, Biochemistry and Biometrics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke B-9820, Belgium

Objectives: To evaluate faecal and clinical scores and presence of several enteropathogens possibly implicated in the development of diarrhoea in pups aged between 6 and 16 weeks independently of their health status.

Methods: Pups were selected from pet shops and breeding facilities and assigned a faecal and clinical score. Standard isolation methods were used to determine presence of parasites, viruses and bacteria in faecal samples. For *Escherichia coli*, virulence genes were assessed by multiplex polymerase chain reaction.

Results: Fifty-six pups were included in this study. Eighteen had no diarrhoea, 22 had no significant clinical signs related to gastroenteritis. Samples were positive for *Toxocara canis* (n=29), *Giardia duodenalis* (n=35), *Cystoisospora* (n=22), *E. coli* (n=47) and *Clostridium perfringens* (n=20). In four *E. coli* positive samples, genes were detected that correlate with pathogenicity in other animal species. A significant positive correlation between the presence of *T. canis* and faecal score was found.

Clinical Significance: Puppies obtained from a pet shop or breeding facility have a high risk of gastrointestinal disease. Furthermore, infectious agents may be present independently of faecal or clinical score. The identification of possible pathogenic *E. coli* strains suggests that their role in diarrhoea warrant further investigation.

Journal of Small Animal Practice (2013) **54**, 475–480
DOI: 10.1111/jsap.12119

Accepted: 25 June 2013

INTRODUCTION

Upon acquiring a pup from a pet shop or breeding facility, gastrointestinal disease is commonly encountered (Hird *et al.* 1992, Scarlett *et al.* 1994). Because most cases of diarrhoea are self-limiting (Steiner 2008, Herstad *et al.* 2010) and specific diagnosis is expensive and difficult to determine (Matz & Guilford 2003), little is known of the specific aetiological agents involved. Indeed, some parasites are difficult to detect because of low sensitivity of the test methods or because of intermittent shedding (Matz & Guilford 2003). Bacteriological examination is rarely performed because of the difficulties in interpretation of the results (Matz & Guilford 2003). Virological diagnostics are hindered by the limited period of faecal shedding, the possibility of false-positive results due to vaccination and the need for highly specialised laboratories for virus isolation (Matz & Guilford 2003).

Therefore, this study was designed to investigate a randomly chosen population of pups aged between 6 and 16 weeks for faecal parasites, viruses and bacteria that may be implicated in diarrhoea. The pups were available for adoption by new owners at pet shops and breeding facilities. All pups were included independently of the presence of diarrhoea and all were assessed for clinical signs correlating with gastroenteritis. In addition, the presence of virulence genes in *Escherichia coli* was examined to determine their pathogenic potential.

MATERIALS AND METHODS

Study design

Samples were collected from pups aged between 6 and 16 weeks, independently of their health status, originating from three breeding facilities, two pet shops and one guide dog training organisation willing to participate.

Each puppy was assigned a body condition score according to the Waltham Size, Health And Physical Examination (S.H.A.P.E.) Score™ containing seven scores from A (underweight) to G (obese) (German *et al.* 2006). Faecal samples were assigned a score ranging from 1 to 7 based on the Purina Faecal Scoring System (Nestlé-Purina Pet Food Co.). To assess the general health status of the pups in relation to gastrointestinal disease, a clinical scoring system was used based on the Canine Inflammatory Bowel Disease Activity Index (CIBDAI) scoring system (Jergens *et al.* 2003). This numerical index uses the presence and frequency of six cardinal gastrointestinal signs to evaluate the degree of illness. The total cumulative scores classify the disease as clinically insignificant (0 to 3), mild (4 to 5), moderate (6 to 8) or severe (9 or greater) gastroenteritis. A questionnaire was taken for each puppy. A breeding facility with less than 10 adult dogs and a pet shop with less than 25 pups were considered small.

Sampling

Fresh faecal samples were collected from each puppy or from one group of pups. If a group sample was positive, every pup of that group was considered positive. Fresh faecal samples and faecal samples collected at the pet shop or breeding facility were transported and stored at 4°C for parasitological analysis within one day. A small amount of fresh faeces was taken for bacteriological analyses and stored at −70°C. If no fresh samples could be obtained, a rectal swab was used. From each puppy another rectal swab was taken for virological examination and placed in transport medium. The extract was collected and stored at −70°C until the time of virological analysis.

Parasitological analysis

Using a sucrose gradient centrifugation-flotation technique (specific density 1·27) and microscopic examination, the presence of *Toxocara canis* eggs and *Cystoisospora* spp. oocysts were determined. The Merifluor direct immunofluorescence assay (IFA, Merifluor Cryptosporidium/Giardia kit; Meridian Diagnostics Inc.) as previously described by Geurden *et al.* (2008) was used to examine the samples for *Giardia* spp. and *Cryptosporidium* spp.

Virological analysis

The extract collected from the rectal swabs was used for virological examination. Isolation of canine parvovirus (CPV) and canine coronavirus (CCoV) was performed on subconfluent and confluent monolayers of A-72 cells, respectively. Canine rotavirus (CRV) isolation was performed on confluent monolayers of MA104. Cells were inoculated for 1 hour with faeces solutions, washed and further incubated for 4 days with culture medium.

Two blind passages were made, the third passage was examined for cytopathic effect and indirect immunofluorescence (IF) was performed to detect CPV, CCoV and CRV. Additionally, hemagglutination activity (HA) of the supernatant was examined for CPV.

Bacteriological analysis

Escherichia coli

An aliquot of faeces was inoculated onto a MacConkey plate and a Columbia Sheep Blood (CSB) plate. After incubation, suspected colonies were subcultured and identified using classical bacteriological methods. *Escherichia coli* was further examined with two multiplex polymerase chain reaction (PCR)'s as previously described (Bruggeman *et al.* 2008), which tested for adhesin factors F4, F5, F6, F18, F41, eae, toxins STb, STa, LT, STx1, STx2, STX2e, CNF1 and CNF2.

Salmonella spp.

An aliquot of faeces was inoculated onto a Brilliant Green Agar plate and incubated for 17 to 24 hours at 37°C. If no colonies indicative of *Salmonella* spp. were found, the plate was incubated for another 24 hours.

Campylobacter spp.

An aliquot of faeces was inoculated onto a modified blood-free charcoal cefoperazone deoxychelate agar (CCDA) plate with a CCDA selective supplement SR0155E (oxoid) and incubated at 37°C in a microaerophilic environment for 48 hours and a successive 48 hours if negative. Suspected colonies were subcultured and identified by PCR. The presence of *Campylobacter coli* and *Campylobacter jejuni* was determined.

Clostridium perfringens

An aliquot of faeces was inoculated onto a CSB plate and a Colisitine Aztreonam plate and incubated anaerobically at 37°C for 18 to 24 hours. Suspected colonies were identified using classical bacteriological methods.

Statistical analysis

The effect of the presence of a particular infectious agent on the faecal score was determined using the multi-nomial model with cumulative logits. In these models, age and type of environment were also incorporated in order to adjust for them. Clinical score 2, a binary variable, is analysed by exact logistic regression, also incorporating age and type of environment. In addition, the correlations were evaluated between the present infectious causes using the correlation matrix.

RESULTS

Pups

Distribution of origin and signalment are shown in Table 1.

Vaccination status

The vaccination status of all the pups was found to be up to date according to the WSAVA guidelines (2010) except for five pups originating from a small pet shop. Furthermore, the vaccination status of the bitches in the breeding facilities were similarly up to date. However, maternal vaccination status was unknown for the other facilities, and for five pups of the small pet shop.

Deworming status

Of the 56 pups, 41 were dewormed less than a month before sampling and 15 pups had an unknown deworming status. The anthelmintics used included fenbendazole (Panacur; MSD Animal Health) (n=9), a combination of fenbendazole and praziquantel (Veprafen; Ceva S.A.) (n=3), pyrantel embonate (Dogminth; Pfizer A.H.) (n=15), a combination of pyrantel embonate, praziquantel and febantel (Drontal; Bayer) (n=10) and a combination of moxidectine and imidacloprid (Advocate spot-on; Bayer HealthCare) (n=5).

Clinical history

The clinical history of the pups revealed that 23 pups had a history of at least one episode of diarrhoea of which one had been confirmed as *Giardia duodenalis* positive and one *Cystoisospora* spp. positive. Of 18 pups, all originating from the pet shops, the clinical history was unknown.

Nutrition

All pups were fed a dry feed. Different brands were used, namely Meradog®, Bento Kronen®, Royal Canin®, Eukanuba® and an in-house brand from a commercial centre. They were fed according to the following frequency: once (n=6), twice (n=19), three times (n=8) or four times (n=1) daily and ad libitum (n=22).

Body condition score (BCS)

According to the Waltham S.H.A.P.E. Score™, 39 of the 56 pups had an ideal body condition score, 6 were mildly overweight, 4 were lean, 2 were thin and 5 pups were extremely thin.

Faecal score

According to the Purina Faecal Scoring System, the following scores were noted: score 2 (n=9), score 3 (n=9), score 4 (n=13), score 5 (n=19), score 6 (n=5) and score 7 (n=1). This corresponds with 18 pups with a stool consistency score of 0, 13 with a score of 1, 19 with a score of 2 and 6 with a score of 3 according to the CIBDAI scoring system (Jergens *et al.* 2003).

Clinical score

After summation of numerical indexes, 22 pups were classified as being healthy or with no clinically significant disease, aged 7 (n=9), 11 (n=1), 12 (n=4), 14 (n=2), 15 (n=2), or 16 (n=2) weeks old and two with ages unknown but between 6 and 16 weeks. Sixteen had mild gastrointestinal disease, aged 7 (n=1) and 12 weeks old (n=15). Two had moderate gastrointestinal disease, both six weeks old. One 6-week old pup had severe gastrointestinal disease. The individual clinical score could not be determined in 15 pups because of group scoring of the faeces.

Housing

The small breeding facility consisted of seven adult dogs. The two larger breeding facilities consisted of 30 and 40 adult dogs. The small pet shop had 22 pups available for sale and the large pet shop had over 300 pups available for sale. The pups of the guide dogs organisation were purchased from breeding facilities and were placed with private owners after 1 week. Every other week, all the pups were assembled for a training session.

All pups of the pet shops and breeding facilities were kept in group housing and all had outdoor excess except those originating from the large pet shop. Of the pups of the dog organisation, two were kept individually. There were no special measures taken in relation to ventilation. All breeding facilities and pet shops removed the faeces at least twice daily. The breeding facilities were cleaned at least once daily using water or a commercial household product. The pet shops only used disinfecting product. Two breeding facilities were disinfected several times weekly. One breeding facility was disinfected once every 2 weeks. They all used chloroxylenol (Dettol®) and one also used sodium hypochlorite (Javel®). The small pet shop was disinfected three times weekly with an industrial product. The large pet shop was disinfected infrequently with an industrial product and sodium hypochlorite (Javel®).

Table 1. Distribution of origin and signalment of 56 puppie

Origin	Breed	Sex	Age
Small breeding facility (n=9)	Dobermann pinscher (n=12)	Female (n=23)	6 weeks (n=3)
Large breeding facilities (n=3 and n=2)	Beagle (n=11)	Male (n=23)	7 weeks (n=10)
Small pet shop (n=20)	Golden retriever (n=4)		9 weeks (n=2)
Large pet shop (n=15)	Cavalier King Charles spaniel (n=4)		10 weeks (n=6)
Guide dog organisation (n=7)	Greater Swiss mountain dog (n=2)		11 weeks (n=1)
	Labrador (n=2)		12 weeks (n=20)
	Rottweiler (n=2)		14 weeks (n=7)
	English bulldog (n=2)		15 weeks (n=3)
	Maltese (n=2)		16 weeks (n=2)
	Crossbreeds (n=5)		Age unknown (n=2)
	Other (n=10)		

Table 2. Results of the statistical analyses for the correlation between the presence of enteropathogens and the presence of diarrhoea and clinically significant score

	Faecal score				Clinical score			
	Number		Median (min,max)		Number		Median (min,max)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
T. canis	27	29	3·0 (2·0, 5·0)	5·0* (2·0, 7·0)	19	22	0·0 (0·0, 0·0)	1·0 (0·0, 1·0)
G. duodenalis	21	35	4·0 (3·0,7·0)	4·0 (2·0, 6·0)	17	24	0·0 (0·0, 1·0)	1·0 (0·0, 1·0)
Cystoisospora spp.	34	22	4·0 (2·0, 5·0)	5·0 (2·0, 7·0)	28	13	0·0 (0·0, 1·0)	1·0* (0·0, 1·0)
E. coli A and B	9	47	4·0 (2·0, 6·0)	4·0 (2·0, 7·0)	7	34	0·0 (0·0, 1·0)	0·0 (0·0, 1·0)
E. coli A	12	44	4·0 (2·0, 6·0)	4·5 (2·0, 7·0)	9	32	0·0 (0·0, 1·0)	0·0 (0·0, 1·0)
E. coli B	52	4	4·0 (2·0, 6·0)	3·5 (2·0, 7·0)	38	3	0·0 (0·0, 1·0)	0·0 (0·0, 1·0)
C. perfringens	36	20	5·0 (2·0, 7·0)	4·0 (2·0, 6·0)	29	12	1·0 (0·0, 1·0)	0·0 (0·0, 1·0)

* Significant correlation

Table 3. Results of the statistical analyses for the correlation between the presence of enteropathogens

	T. canis	*G. duodenalis*	*Cystoisospora* spp.	*C. perfringens*	*E. coli*
T. canis	1·000	0·49264*	0·35996*	−0·38799*	−0·03286
G. duodenalis	0·49264*	1·000	0·24403	−0·35916*	−0·12471
Cystoisospora spp.	0·35996*	0·24403	1·000	−0·29419*	0·15273
C. perfringens	−0·38799*	−0·35916*	−0·29419*	1·000	0·11055
E. coli	−0·03286	−0·12471	0·15273	0·11055	1·000

* Significant correlation

All the facilities had a history of clinical problems with *G. duodenalis*, one had a history with *Cystoisospora* spp. and CPV, one with *T. canis* and one with CPV. These had occurred less than 2 years ago.

Laboratory findings

There were 19 individual fresh faecal samples and 14 group samples collected. The following potential enteropathogens were detected: *T. canis* (n=29) (individual samples: n=2; group samples: n=9); *G. duodenalis* (n=35) (individual samples: n=6; group samples: n=11); *Cystoisospora* spp. (n=22) (individual samples: n=1; group samples: n=8); *E. coli* (n=47) and *C. perfringens* (n=20).

All samples were negative for *Salmonella* spp. PCR showed that three *E. coli* strains contained *STa*-positive genes and one sample was positive for both *STa* and *STb* genes. *C. jejuni* was found in one sample that consisted of the faeces of three pups held in one group. Three samples were HA-positive for CPV, but not confirmed with IF. All other test results were negative. In only one puppy no potential enteropathogens were detected.

Statistical evaluation

The effect of the presence of a particular enteropathogen on the faecal score according to the Purina Faecal Scoring system and the CIBDAI score is shown in Table 2. The CIBDAI score has been divided into the absence or presence of clinically significant signs of gastroenteritis. The statistical analysis showed a significant correlation between the presence of *T. canis* and the faecal score. There was also a significant correlation between the presence of the *Cystoisospira* spp. and the clinical score.

Further, there was a significant correlation between the presence of *T. canis* and the presence of *G. duodenalis* and *Cystoisospora* spp., as shown in Table 3.

DISCUSSION

In this study, a population of pups during their critical period of transition (Davis-Wurzler 2006) in which diarrhoea poses an important problem were targeted (Yeşilbağ *et al.* 2007). To the authors' knowledge, no previous studies have examined dogs in this age group for a wide variety of enteropathogens, combined with the use of objective parameters to score the health status.

Overall, possible infectious agents were detected in all but one pup. This may be an overestimation as pups were considered positive if the group sample was positive for parasitological analyses. However, it is highly likely that if one pup in a group is infected by parasites, the other pups are also infected. The most common isolated parasitic enteropathogen was *G. duodenalis*, followed by *T. canis* and *Cystoisospora* spp. This is in accordance with a recent parasitic study in the Belgian dog population (Claerebout *et al.* 2009). This result might have been expected as dogs, younger than six months old and living in large groups, have an increased risk of contracting a parasitic infection (Claerebout *et al.* 2009, Gates *et al.* 2009). It is, however, possible that the presence of some pathogens is underestimated. For example, the sensitivity of faecal examination for detection of *G. duodenalis* by means of IFA is over 90% (Geurden *et al.* 2008), but because of intermittent excretion some infected animals may have been overlooked (Epe *et al.* 2010). *Toxocara canis* is a well-known parasite in pet shops and breeding facilities and regular deworming schedules were maintained. All pups, of which the deworming status was known, were dewormed less than a month before sampling. However, half of the sampled population was positive for *T. canis*. This could also be an underestimation as only the adult *T. canis* worms are detected and not the migrating larvae. A plausible explanation is that the hygiene measures are inadequate to avoid reinfection. Indeed, the eggs and oocysts of the parasites are very resistant and a correct disinfectant and frequent cleaning are of primary

importance to decrease the infection pressure (Overgaauw 1997). This clearly could be improved in some of the facilities. Also the used antihelminthics are not effective against larval stages.

The technique used for virological analysis relies on the cytopathic effect of the viruses, which may have been lost during handling of the samples. The lack of evidence of the presence of canine enteric viruses, as opposed to the high seroprevalence rates of especially CCoV which can increase from 44% up to 100% in pups (Tennant *et al.* 1993, Bandai *et al.* 1999, Squires 2003) may be explained by the age of the pups sampled. A similar prevalence of approximately 40% was detected in faecal samples in other studies, with 8 of 10 positive samples originating from Belgium (Naylor *et al.* 2001, Schulz *et al.* 2008, Decaro *et al.* 2009).

The absence of CPV-positive samples was not unexpected because of the low number of pups with a clinical score corresponding with a moderate or severe gastroenteritis. CRV was also not detected in the faecal samples. This is in accordance with the findings of Yeşilbağ *et al.* (2007) and the low prevalence of 2·4% in Japan (Mochizuki *et al.* 2001).

The most important bacterial enteropathogens in dogs are *Salmonella* spp., *Campylobacter* spp., enteropathogenic and enterotoxigenic *E. coli* and *C. perfringens* (Batt *et al.* 1996, Cave *et al.* 2002, Marks & Kather 2003, Hall 2004, Steiner 2008). Of these *Salmonella* spp. and *Campylobacter* spp. are the most frequently isolated. *Escherichia coli* and *C. perfringens* can easily be found because they are part of the normal intestinal microflora and only after detecting virulence genes, these strains may be suspected to be implicated in the development of diarrhoea. The interpretation of these results is therefore difficult (Hall 2004). There is, however, growing evidence that certain *E. coli* strains may cause intestinal disease in dogs (Beutin *et al.* 1993, Hammermueller *et al.* 1995, Starčič *et al.* 2002). Actually, no unambiguous differentiation is possible between non-pathogenic and pathogenic strains. In this study, a very small number of samples contained genes known to be present in Enterotoxigenic *E. coli* (ETEC) and code for the production of enterotoxins. It is remarkable that the only sample containing *STa* as well as *STb* genes originated from a pup with severe diarrhoea. However, because of simultaneous parasitic infections, no clear conclusions could be made.

Salmonella spp. and *Campylobacter* spp. were uncommon. However, samples were frozen at –70°C before analysis and this might have reduced the sensitivity for *Campylobacter* detection. The sensitivity of the detection methods used to examine faecal samples for *Salmonella* spp. and *Campylobacter* spp. were adequate to diagnose clinical cases but not carrier status.

Because of the use of commercial diets, the prevalence is expected to be low as dogs receiving a natural diet have a higher prevalence of *Salmonella* spp. (Green 2006). The one group that showed severe diarrhoea and pot-belly appearance, typical for *Campylobacter* infection, was positive for *C. jejuni*. However, these pups were also severely infected with *T. canis*, which may reduce the role of *C. jejuni* to that of a secondary one. *Clostridium perfringens* was commonly isolated but in small numbers per sample and therefore was not clinically significant.

There was only a significant correlation between the presence of *T. canis* and the faecal score. This means that a normal faecal or clinical score does not preclude the presence of enteropathogens. Furthermore, there were also correlations found between the presence of *T. canis* and *G. duodenalis* and *Cystoisospora* spp. As in all three parasites hygiene is an important part of the prevention, this was expected.

Previous studies have demonstrated that a low BCS increases the risk for developing diarrhoea, severe illness and death (Scarlett & Donoghue 1998, Doria-Rose & Scarlett 2000). A small number of pups had a precarious BCS, but the largest part of the population was in a good condition. However, only 18 pups did not have diarrhoea and the evaluation of the clinical score showed that more than half had a mild to severe gastroenteritis. The history of diarrhoea did not appear to have an effect on the classification of the pups in the different groups. Worrisome is that all pups, except those with severe diarrhoea, were considered by the owners of being without gastroenteritis and were available for purchase (data not shown). The information on the clinical history supplied by the owners may not therefore be reliable.

All pups were fed dry rations, therefore there was no confounding factor of wet feed that could have an influence on faecal scoring. However, presumably the choice of brand could have an effect on faecal consistency. Some brands have been shown to have a better digestibility leading to better faecal consistency (Brambillasca *et al.* 2010). Another possible effect could be the presence of glycoprotein-containing ingredients like eggs, which have been suggested to have a protective effect against pathogenic adhesive *E. coli*. This is, however, a field of study which warrants additional research.

As possible enteropathogens were detected in all but one puppy, further research is warranted to be able to assign the true pathogen to the diarrhoea seen. Moreover, further studies are required to determine the role of the isolated *E. coli* strains. Practitioners should be aware that diarrhoea is not only a common but also often unrecognised problem in pups. More importantly, the absence of clinical signs of gastroenteritis should not preclude the absence of enteropathogens. Secondly, only half of the pups in this population, of which a clinical score could be determined, were without clinical signs of gastroenteritis. Indeed, new pet owners should have their newly purchased puppy examined by a veterinary surgeon, even if they seem to be in good health. Specific attention should be given to basic hygiene, especially with children, to prevent zoonotic infection.

Acknowledgements

This study was supported by Veos N.V. and the agency for Innovation by Science and Technology (IWT). We thank Andy Luccina for his excellent technical assistance.

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.

References

Bandai, C., Ishiguro, S., Masuya, N., *et al.* (1999) Canine coronavirus infections in Japan: virological and epidemiological aspects. *Journal of Veterinary Medical Science* **61**, 731-736

Batt, R. M., Rutgers, H. C. & Sancak, A. A. (1996) Enteric bacteria: friend or foe? *Journal of Small Animal Practice* **37**, 261-267
Beutin, L., Geier, D., Steinrück, H., *et al.* (1993) Prevalence and some properties of verotoxin (Shiga-like toxin)-producing Escherichia coli in seven different species of healthy domestic animals. *Journal of Clinical Microbiology* **31**, 2483-2488
Brambillasca, S., Purtscher, F., Britos, A., *et al.* (2010) Digestibility, fecal characteristics, and plasma glucose and urea in dogs fed a commercial dog food once or three times daily. *Canine Veterinary Journal* **51**, 190-194
Bruggeman, M., Decostere, A., Pasmans, F., *et al.* (2008) Zin en onzin van het onderzoek van fecesstalen van honden met diarree op de aanwezigheid van Escherichia coli. *Vlaams Diergeneeskundig Tijdschrift* **78**, 177-181
Cave, N. J., Marks, S. L., Kass, P. H., *et al.* (2002) Evaluation of a routine diagnostic fecal penal for dogs with diarrhea. *Journal of the American Veterinary Medical Association* **221**, 52-59
Claerebout, E., Caseart, S., Dalemans, A.-C., *et al.* (2009) Giardia and other intestinal parasites in different dog populations in Northern Belgium. *Veterinary Parasitology* **161**, 41-46
Davis-Wurzler, G. M. (2006) Current vaccinations strategies in puppies and kittens. *Veterinary Clinics of Small Animals* **36**, 607-640
Decaro, N., Desario, C., Billi, M., *et al.* (2009) Western European epidemiological survey for parvovirus and coronavirus infections in dogs. *The Veterinary Journal* **187**, 195-199
Doria-Rose, V. P. & Scarlett, J. M. (2000) Mortality rates and causes of death among emanciated cats. *Journal of the American Veterinary Medicine Association* **216**, 347-351
Epe, C., Rehkter, G., Schnieder, T., *et al.* (2010) Giardia in symptomatic dogs and cats in Europe – results of a European study. *Veterinary Parasitology* **173**, 32-38
Gates, M. C. & Nolan, T. J. (2009) Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Veterinary Parasitology* **166**, 153-158.
German, A. J., Holden, S. L., Moxham G. L., *et al.* (2006) A simple, reliable tool for owners to assess the body condition of their dog or cat. *The Journal of Nutrition* **136**, 2031S-2033S
Geurden, T., Berkvens, D., Casaert, S., *et al.* (2008) A Bayesian evaluation of three diagnostic assays for the detection of Giardia duodenalis in symptomatic and asymptomatic dogs. *Veterinary Parasitology*, **157**, 14-20
Green, E. (2006) Enteric bacterial infections, canine viral enteritis. In: Infectious Diseases in the Dog and Cat. Elsevier, St. Louis, MO, USA
Hackett, T. & Lappin, M. R. (2003) Prevalence of enteric pathogens in dogs of north-central Colorado. *Journal of the American Animal Hospital Association* **39**, 52-56
Hall E. J. (2004) Bacterial enteropathogens in dogs. Proceedings of the 29th World Congress of the WSAVA. Rhodes, Greece, October 6-9, 2004
Hammermueller, J., Kruth, S., Prescott, J., *et al.* (1995) Detection of toxin genes in Escherichia coli isolated from normal dogs and dogs with diarrhea. *Canadian Journal of Veterinary Research* **59**, 265-270
Herstad, H. K., Nesheim, B. B., L'Abéé-Lund, T., *et al.* (2010) Effects of a probiotic intervention in acute canine gastroenteritis – a controlled clinical trial. *Journal of Small Animal Practice* **51**, 34-38
Hird, D. W., Ruble, R. P., Reagor, S. G., *et al.* (1992) Morbidity and mortality in pups from pet stores and private sources: 968 cases (1987-1988). *Journal of the American Veterinary Medicine Association* **201**, 471-474
Jergens, A. E., Schreiner, C. A., Frank, D. E., *et al.* (2003) A scoring index for disease activity in Canine Inflammatory Bowel Disease. *Journal of Veterinary Internal Medicine* **17**, 291-297
Marks, S. L. & Kather, E. J.(2003) Bacterial-associated diarrhea in the dog: a critical appraisal. *Veterinary Clinical of North America: Small Animal Practice* **33**, 1029-1060
Matz, M. E. & Guilford, W. G. (2003) Laboratory procedures for the diagnosis of gastrointestinal tract disease of dogs and cats. *New Zealand Veterinary Journal* **51**, 292-301
Mochizuki, M., Hashimoto, M. & Ishida, T. (2001) Recent epidemiological status of canine viral enteric infections and Giardia infection in Japan. *Journal of Veterinary Medical Science* **63**, 573-575.
Naylor, M. J., Naylor, M. J., Monckton, R. P., *et al.* (2001) Canine coronavirus in Australian dogs. *Australian Veterinary Journal* **79**, 116-119
Overgaauw, P. A. M. (1997) Aspects of Toxocara epidemiology: Toxocarosis in dogs and cats. *Critical Review in Mircrobiology* **23**, 233-251
Scarlett, J. M. & Donoghue, S. (1998) Associations between body condition and disease in cats. *Journal of the American Veterinary Medicine Association* **212**, 1725-1731
Scarlett, J. M., Saidla, J. E. & Pollock, R. V. (1994) Source of acquisition as a risk factor for disease and death in pups. *Journal of the American Veterinary Medicine Association* **204**, 1906-1913
Schulz, B. S., Strauch, C., Mueller, R. S., *et al.* (2008) Comparison of the prevalence of enteric viruses in healthy dogs and those with acute hemorrhagic diarrhoea by electron microscopy. *Journal of Small Animal Practice* **49**, 84-88
Squires, R. A. (2003) An update on aspects of viral gastrointestinal diseases of dogs and cats. *New Zealand Veterinary Journal* **51**, 252-261
Starčič, M., Johnson, J. R., Stell, A. L., *et al.* (2002) Haemolytic Escherichia coli isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxigenic strains. *Veterinary Microbiology* **85**, 361-377
Steiner, J. M. (2008) Small Animal Gastroenterology. Schlutersche Verlagsgesellschaft mbH& Co. KG, Hannover, Germany.
Tennant, B. J., Gaskell, R. M., Jones, R. C., *et al.* (1993) Studies on the epizootiology of canine coronavirus. *Veterinary Record* **1**, 7-11.
Vaccination Guidelines Group, Day, M. J., Horzinek, M. C., Schultz, R. D. (2010) WSAVA guidelines for the vaccination of dogs and cats. *Journal of Small Animal Practice* **51**, 1-32
Yeşilbağ, K., Yilmaz, Z., Ozkul, A., *et al.* (2007) Aetiological role of viruses in puppies with diarrhoea. *Veterinary Record* **161**, 169-170