Global Veterinaria 13 (3): 302-307, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.03.84284

Canine Viral Diarrhea: Clinical, Hematologic and Biochemical Alterations with Particular Reference to In-Clinic Rapid Diagnosis

N.Y. Salem

Department of Internal Medicine and Infectious Diseases,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Diarrhea is one of most faced medical problems in canine practice; viral causes comprise a good proportion. Thirty-five diarrheic dogs examined in this study for clinical signs, hematologic, serum biochemical alterations; rapid in-clinic immune chromatography test used to determine the causative agents and results were compared to RT-PCR findings. Anemia, leucopenia, neutropenia and lymphopenia were the most observed hematologic alterations in both viruses, increase in ALT, ALP, triglycerides with decrease in albumin, sodium and chloride were the most observed serum biochemical alterations in CPV while increase in BUN and triglycerides and decrease in sodium level observed in CDV. Immune chromatography [*IC*] test is a rapid, sensitive and accurate tool for in-clinic diagnosis for both CPV and CDV antigen and when compared with RT-PCR for CDV.

Key words: CPV · CDV · Hematology · Biochemistry · IC

INTRODUCTION

In canine practice, diarrhea is a common complaint faced by canine medical practitioners daily [1], it is a multi-factorial condition, the etiologic agents include diet, parasites and infectious agents [2], viral causes as canine parvovirus (CPV) and canine Distemper virus (CDV) comprise a part of this problem.

CPV-2 is a common cause of acute hemorrhagic enteritis in dogs; it is small non-enveloped DNA virus [3]. Anorexia, depression, fever, vomiting and small bowel diarrhea ranged from mucoid to hemorrhagic and dehydration are the most consistent clinical signs associated with CPV enteritis [4].

CDV is a member of *paramyxoviridae* that cause severe multi-systemic fatal illness [5], the clinical presentations correlated essentially to respiratory, gastrointestinal (of which the diarrhea) and central nervous system [6, 7]. Distemper virus has the second high fatality rate compared to any infectious disease; it came after the rabies in domestic dogs [8].

Few papers discussed the hematological and biochemical alterations in CPV and CDV infected dogs. Panleucopenia, neutropenia, lymphopenia and anemia [9-11] along with hypoalbuminemia, hypoproteniemia and increase in serum triglycerides concentrations [11, 12] are the frequent abnormalities observed in CPV infection. Lymphopenia with leucopenia or leucocytosis and anemia along with hypoalbuminemia and elevated BUN are the characteristic alterations associated with CDV infection [13, 14].

Many diagnostic techniques developed for rapid detection of offending organism, of which immune chromatography. Immune chromatography (IC) test has developed as rapid in-clinic diagnostic tool used in clinical practice for quick detection of CDV and CPV antigens [15, 16]. RT-PCR remains fast, sensitive and specific method for diagnosis of CDV infection in dogs [17], but the availability of the utility limits its usage.

This study designed to inspect the clinical, hematologic, clinic- pathological alterations associated with CDV and CPV infected dogs with special reference to immune chromatography as rapid in-clinic diagnostic tool.

MATERIALS AND METHODS

A total number of 35 diarrheic dogs used in this study, dogs referred to Small Animal Medicine

Corresponding Auther: Noha Yousef Salem, Department of Internal Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt, Tel: +202-01005431215.

Teaching Hospital, Faculty of Veterinary Medicine, Cairo University, clinical signs recorded at the time of admission, complete examination, clinical hematology performed.

Sera of infected animals analyzed for ALT, AST, total protein, albumin, bun, creatinine, triglycerides, sodium and chloride (Stanbio, Texas, USA), ALP and total bilirubin (QCA, Tarrogona, Spain) using spectrophotometer (APEL, PD-303S, Japan).

Immunochromatographic assay was performed for both CDV and CPV antigen detection using commercial test kits (Canine Parvo Virus Ag Rapid Test Kit by Immunochromatographic Assay and One-step Canine distemper virus Ag test, Quicking Biotech Co, Ltd, Shanghai, China) according to manufacturer instructions.

RNA was extracted from fresh whole using QIAamp RNA blood mini kit (QIAGEN) according to manufacturer's instructions. Primers that amplify canine distemper nucleoprotein (NP) gene sequences [17, 18] Table 1, expected amplicons lengths are 260 and 287bp respectively. RT-PCR was performed according to the method described by Frisk *et al.* [17].

RESULTS

The most consistent clinical signs observed in CPV-infected dogs were fever (17/24), bloody diarrhea (17/24), diarrhea (7/24), vomiting (12/24) and dehydration, while the most consistent clinical signs observed in CDV-infected dogs were fever (10/11), diarrhea (11/11), respiratory signs (11/11), vomiting (6/11) and dehydration.

The hematologic results showed a decrease in RBCs count, hemoglobin content, PCV percentage, leucocytes count, neutrophil and lymphocyte in both CPV-infected dogs and CDV- infected Dogs when compared to normal reference ranges as showed in Table 2.

Serum biochemical analysis showed a decrease in albumin concentration with increased activities of ALT and ALP, bilirubin, triglycerides and BUN along with a decrease in sodium and chloride in CPV-infected dogs. Serum biochemical alterations in CDV-infected dogs showed an increase in triglyceride and BUN concentration along with decrease in sodium level when compared to normal reference ranges as showed in Table 2.

Table 1: Primers used for RT-PCR

Sequence 5'-3'	Direction	Expected amplicon length
AAC TAT GTA TCC GGC TCT TGG	Sense	260bp
CGA GTC TGA AGT AAG CTG GGT	Antisense	
ACA GGA TTG CTG AGG ACC TAT	Sense	287bp
CAA GAT AAC CAT GTA CGG TGC	Antisense	

Primers that amplify canine distemper nucleoprotein (NP) gene sequences according to Frisk *et al*. [17] and Sidhu *et al*. [18]

Table 2. Hematologic and serum biochemical findings of CPV and CDV infected dogs

	CPV infected dogs	CDV infected dogs	
Parameter	Mean ±SEM	Mean ±SEM	International Reference range*
RBCs $\times 10^6$/il	4.0341±0.31	4.26±0.334	5.5 – 8.5
Hemoglobin g/dl	11.939±0.52	10.47±1.394	12.0 – 18.0
PCV %	36.118±1.404	31.70±3.31	37 – 55
WBCs $\times 10^3$/il	3.935±0.289	4.054±0.81	6,000 – 17,000
Neutrophil $\times 10^3$/il	2.675±0.321	2.878±0.201	3,000 – 11,500
Lymphocyte $\times 10^3$/il	0.865±0.129	0.729±0.112	1,000 – 4,800
Total Protein g/dl	5.797±0.37	6.61±1.64	5.3–7.6
Albumin g/dl	2.54±0.21	3.53±1.12	3.2–4.7
Globulin	3.257±0.68	3.08±0.57	1.5–3.5
ALT u/l	99.37±14.04	65.36±16.41	10–94
AST u/l	59.47±13.9	59.98±11.7	10–62
ALP u/l	194.61±15.7	107.30±14.28	20-156
Cholesterol mg/dl	211.160±32.8	213.18±14.8	135-270
Triglycerides mg/dl	173.67±12.8	178.22±16.3	20-112
Total bilirubin mg/dl	0.73±0.34	0.343±0.124	0.1–0.6
BUN mg/dl	32.62±8.55	30.58±6.59	10–28
Creatinine mg/dl	0.882±0.322	1.22±0.231	0.5–1.4
Sodium mEq/L	138.69±5.32	140.3±4.18	141-152
Chloride mEq/L	90.235±15.6	114.73±21.3	105-115

*International reference range according to Tvedten [19], Kaneko *et al*. [20] and Rizzi *et al.* [21].

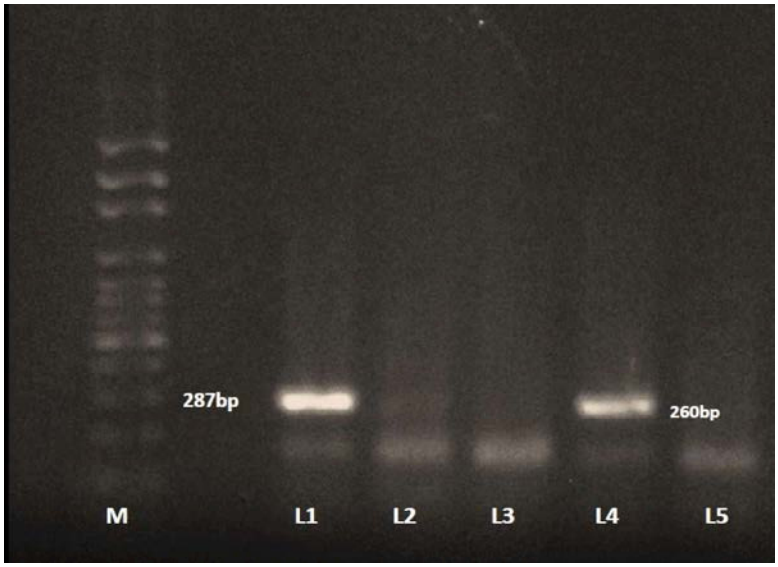

Photo 1: Analysis of Reverse transcription Polymerase Chain reaction (RT- PCR) products showing the positive samples at260 and 287bp for canine distemper virus.

IC test was able to detect CDV antigen in (10/35) samples and CPV antigen on (24/35) samples. The confirmation of the diagnosis performed based on RT-PCR dedicated for CDV using two different primers, both 260 and 287 bp amplicon (photo 1) detected in (11/35) samples.

DISCUSSION

Diarrhea is a common manifestation clinical practitioner's deal with daily, numerous viruses are implicated as the cause of diarrhea in dogs; primary intestinal viruses of dogs are CPV and CDV [1]. A constellation of signs observed in CPV infection, Goddard and Leisewitz [22] found although the signs are non-specific, but it may form the basis of preliminary diagnosis. Pro-inflammatory cytokines and endotoxin may potentiate a systemic inflammatory response [23].

A pantropic signs nature of CDV infection observed, CDV infects the macrophages and lymphocytes which carries the virus to surface epithelium of respiratory, digestive and nervous system with clinical signs ensue [6].

An observed decrease in RBCs, HB and PCV values in CPV-infected dogs, a virus suppression of bone marrow along with alterations in erthroid, myloid and megakaryocyte can cause these alterations [24]. Wazir *et al*.[25] attributed the reduction in erythrogram to intestinal hemorrhage.

A decrease in Leucocytes, neutrophils and lymphocytes compared to the normal reference ranges were found in CPV infection, the destruction of rapidly dividing cells along with massive neutrophil exudation into damaged gut [26], the destruction of mitotically active precursors of circulating leucocytes especially neutrophils could be involved [27].

An observed decrease in PCV, HB and RBCs count in CDV-infected dogs compared to normal reference range. A proposed erythroid hypoplasia by the virus has been suggested [28] or production of inflammatory mediators that can inhibit the erythropioisis and decrease the RBCs life span [29]. A decrease in leucocytes with subsequent decrease in neutrophils and lymphocytes was found, leucopenia is a primary lymphopenia due to viral damage of lymphoid cells [6], the viral multiplication in lymph nodes is responsible for lymphopenia and subsequent leucopenia along with lymphoid depletion, necrosis, apoptosis [30] and loss of lymphocyte proliferation ability [7].

An observed decrease in albumin, sodium and chloride levels along with observed increase in ALT, ALP, bilirubin, triglycerides and BUN in CPV-infected dogs when compared to normal reference ranges, the puppies with CPV enteritis may develop severe protein losing enteropathy due to intestinal villi damage [31] or intestinal hemorrhage [19]. Serum enzymes AST, ALT and ALP showed non-significant increase compared to healthy control [25].

The increase in triglycerides levels is thought to be mediated by acute phase response accompanying lipid metabolism alterations [32]. The dehydration associated with the illness can cause an elevation in BUN concentration [33]. The gastrointestinal lesion, intestinal loss in diarrhea or loss in vomiting are responsible for electrolyte abnormalities [34].

An increase in triglyceride and BUN in CDV with decrease in sodium levels compared to reference ranges were observed, measurement of triglycerides is indicated if clinical signs associated with hypertriglycerdiemia are exist (*i.e*. seizures and gastrointestinal signs) [35]; the increased mobilization to serum from liver may reflect thomeostasis attempts following viral influences on lipid metabolism [36]. The most consistent alterations in lipoprotein metabolism during infection and inflammation are hypertriglycerdaemia [19]. The decrease in tissue perfusion and dehydration may lead to increase in BUN levels [33]. Non-renal losses of sodium may be gastrointestinal (e.g. vomiting and diarrhea) leading to hyponatermia [37].

Rapid in-clinic diagnostic tools used in clinical practice for quick detection of CDV and CPV antigens have been appraised [15, 16]. The immunochromatography proved to be accurate, quick, sensitive and specific [38], the importance of the rapid diagnosis of CPV-2 infection especially in kennels and shelters for isolation of infected dogs and since the clinical diagnosis is not conclusive, IC is the most common rapid diagnostic tool [39]. Lateral flow immunoassay is suitable format as frontline diagnosis of CPV [40].

In CDV, the *IC* test has many advantages over other field diagnostic tests used in clinical practice, the test is simple and rapid [16, 41]. The early detection would permit appropriate treatment and quarantine to be established quickly which help in reducing morbidity, mortality and spread to other dogs [42]. Although RT-PCR for CDV is stoutly influenced by selected primers but it remains the highly specific and sensitive methods for ante mortem diagnosis [43, 44]. *IC* assay is slightly less sensitive and specific than RT-PCR but it remains more rapid, readily available and not need special instruments [16, 42, 43].

CONCLUSION

The hematologic and biochemical alterations associated with CPV and CDV infections may diverge but anemia, leucopenia, neutropenia and lymphopenia are the most observed hematologic alterations in both viruses, increase in ALT, ALP, triglycerides with decrease in albumin, sodium and chloride are the most observed serum biochemical alterations in CPV. Few biochemical abnormalities detected in CDV including increase in BUN and triglycerides and decrease in sodium level. *IC* test is a rapid, sensitive and accurate tool for in-clinic diagnosis for CDV and CPV antigen and when compared with RT-PCR for CDV, it showed nearly similar results.

ACKNOWLEDGEMENT

The author like to express thanks to Dr. Mohammed Abd Elmohsen Elshahaat, assistant lecturer of virology, faculty of veterinary medicine, Cairo University for his valuable assist regarding RT-PCR technique and Professor Taher A Baraka for his valuable aid in improvement of this paper.

REFERENCES

1. Tams, T.R., 2003. Gastrointestinal symptoms. In: Handbook of Small Animal Gastroenterology, Eds., Tams, T.R., 2nd Edn.), Elsevier science, USA.
2. Willard, M.D., 2009. Digestive System Disorders. In: Small Animal Internal Medicine, Eds., Nelson, R.W. and C.C. Couto, 4th edn., Mosby, Elsevier, St. Louis, Missori, pp: 351-486.
3. Carmichael, L.E. and L.N. Binn, 1981. New enteric virus in dog. Adv. Vet. Sci. Comp. Med., 25: 1-27.
4. Goddard, A., A.L. Leisewtz, M.M. Chistopher, N.M. Duncan and P.J. Becker, 2008. Prognostic usefulness of blood leukocyte changes in canine parvo viral enteritis. J. Vet. Int. Med., 22(2): 309-316.
5. Greene, C.E., 2008. Canine Distemper: what's new in treatment and prevention? In International congress of Italian association of companion animal veterinarian, Rimini, Italy, May30-June, 2008, 1: 229-230.
6. Greene, C.E. and M.J. Appel, 2006. Canine Distemper. In: Infectious Diseases of the Dogs and Cats, Eds., Greene, C.E, 3rd edn., Elsevier.
7. Pratelli, A., 2011. Canine Distemper Virus the emergence of new Variants. Vet. J., 187: 290-291.
8. Deem, S.L., L.H. Spleman, R.A. Yates and R.J. Montal, 2000. Canine Distemper in terrestrial carnivores: A review. J. Zoo and Wildlife Medicine, 31(4): 441-451.

9. Zafar, M.S., S.A. Khan and A. Rabbani, 1999. Hematological studies and estimation of electrolytes in dogs exhibiting diarrhoeal signs. Pakist. Vet. J., 19(1): 35-39.
10. Haligur, M., O. Ozman, K. Sezer and S. Sahindurams, 2009. Clinical, pathological and immunohistochemical findings in diarrheic dogs and evaluation of canine parvovirus and corona viral enteritis. Journal of animal and veterinary advances, 8(4): 720-725.
11. Tabor, B., 2011. Canine Parvovirus. Comp. Cont. Educ. Vet. Tech., May 2011, pp: 1-9. https://s3.amazonaws.com/ assets. prod.vetlearn.com/ 2a/c3dfa0a9a611e 087120050568d3693/ file/VT0511_Tabor_CE.pdf.
12. Yilmaz, Z. and S. Senturk, 2007. Characterization of lipid profiles in dogs with parvovirus enteritis. Journal of small animal practice, 48: 643-650.
13. Appel, M.J., R.A. Yates, G.L. Foley, J.J. Bernstein, S. Santinelli, L.H. Spelman, L.D. Miller, L.H. Arp., M. Anderson, Barr, S. Pearce-Kelling and B.A. Summers, 1994. Canine distemper epizootic in lions, tigers and leopards in North America. J. Vet. Diagn. Invest., 6: 277-288.
14. Almeida, R.K., A.C. Vasconcelos, R.A. Carneiro, P.R.O. Paes and L. Moro, 2009. Cytological alterations of the bone marrow and peripheral blood of dogs with canine distemper. Arq. Bras. Med. Vet. Zootec., 61(6): 1255-1260.
15. Esfandiari, J. and B. Klingerborn, 2000. Comparative study of new rapid and one-step test for detection of parvovirus in feces from dogs, cats and mink. J. Vet. Med. B. Infect. Dis. Vet. Public Health, 47(2): 145-153.
16. An, D., K. Tae-Young, S. Daesub, K. Bo-Kyu and P. Bong-Kyun, 2008. Immunochromatography assay for rapid Antemortem diagnosis of dogs suspected to have canine distemper. Journal of Virological Method, 147(2): 244-249.
17. Frisk, A.L., M. König, A. Moritz and W. Baumgärtner, 1999. Detection of canine distemper virus nucleoprotein RNA by reverse transcription PCR using serum, whole blood and cerebrospinal fluid from dogs with distemper. Journal of Clinical Microbiology, 37(11): 3634-3643.
18. Sidhu, M.S., W. Husar, S.D. Cook, P.C. Dowling and S.A. Udem, 1993. Canine distemper terminal and intergenic non-protein coding nucleotide sequences: completion of the entire CDV genome sequence. Virology, 193: 66-72.
19. Tvedten, H., 2004. Appendix II. In: Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H. Tvedten, 4th edn., Saunders. USA, pp: 417-419.
20. Kaneko, J.J., J.W. Harvey and M. Bruss, 2008. Veterinary Clinical Biochemistry of Domestic Animal. 6th edn., Elsevier, pp: 889-895.
21. Rizzi, T.E., J.H. Meinkoth, K.D. Clinkenbeard, 2010. Normal hematology of the dogs. In Schalm's Veterinary hematology, Eds., Weiss D.J. and K.J. Wardrop, Weiley-Blackwell publishing Ltd., pp: 799-810.
22. Goddard, A. and A.L. Leisewitz, 2010. Canine Parvovirus. Vet Clin Small Anim, 40: 1041-1053.
23. Weiss, D.J. and J. Rashid, 1998. The sepsis-coagulant axis. J. Vet. Int. Med., 12(5): 317-324.
24. Potgieter, L.N.D., J.B. Jone, G.S. Patton and T.A. Webb-Martin, 1981. Experimental Parvovirus infection in dogs. Can. J. Comp. Med., 45: 212-216.
25. Wazir, V.S., S.K. Gupta, R.K. Bhardwaj, A.K. Taku, J.S. Soodan, S.K. Kotwal, R. Singh and V.P. Singh, 2011. clinic-hematological alterations in CPV gastroenteritis in canines. In Proceedings of 29th ISVM Convention and National Symposium on "Recent Developments in Diagnostics and Therapeutics Including Applications of Nano-Technology in Veterinary Medicine". 17th-19th February 2011.
26. Raskins, R.E., K.S. Latimer and H. Tvedton, 2004. Leucocyte disorder. In Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H. Tvedten, 4th edn., Saunders.
27. McCaw, D.L. and J.D. Hoskins, 2006. Canine parvovirus. In Infectious Diseases of the Dogs and Cats, Eds., Greene, C.E., 3rd edn., Elsevier, pp: 63-73.
28. Ezeib, M.C.O. and R.I. Udegbunam, 2008. Haematology of dogs infected with canine distemper virus. Sokoto J. Vet. Science, 7(2): 31-33.
29. Meyer, D.J. and J.W. Harvey, 1998. Veterinary Laboratory Medicine: Interpretation and diagnosis, 2nd Ed., W.B. Saunders, pp: 43-82.
30. Pardo, I.P.R., 2006. Phylogenetic characterization of canine distemper virus detection in naturally infected North American dogs. PhD. thesis, Graduate school of University of Missouri-Cloumbia, Missouri, USA.
31. Prittie, J., 2004. Canine parvovirus enteritis: a review of diagnosis, management and prevention. J of veterinary emergency and critical care, 14(3): 167-176.

32. Hudgins, L.C., T.S. Parker, D.M. Levine, B.R. Gordon, S.D. Saal, X.C. Jiang, C.E. Seidman, J.D. Tremaroli, J. Lai and A.L. Rubin, 2003. A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers. Journal of Lipid Research, 44: 489-498.
33. Barsanti, J.A., G.E. Lees, M.D. Willard and R.A. Green, 2004. Urinary disorders. In Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H. Tvedten, 4th edn., Saunders.
34. Macintire, D.K. and S. Smith-Carr, 1997. Canine parvovirus. Part II: clinical signs, diagnosis and treatment. Comp. Cont. Edu. Pract. Vet., 19: 291-302.
35. Nelson, R.W., G.H. Turnwald and M.D. Willard, 2004. Endocrine, Metabolic and Lipid disorders. In Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H, Tvedten, 4th edn., Saunders.
36. Bresnahan, M.R. and P.M. Newberne, 1968. interaction of diet and distemper virus infection on lipid metabolism in the dogs. Br. J. Exp. pathol., 49(3): 223-234.
37. Tvedten H., 2004. Appendix II. In: Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H. Tvedten, 4th edn., Saunders. USA, pp: 417-419.
38. Berbee, J.F., L.M. Havekes and P.C. Rensen, 2005. Apolipoproteins modulate the inflammatory response to lipopolysaccharide. Journal of Endotoxin Research, 11: 97-103.
39. Dibartola, S.P., R.A. Green, H.S. Autran de Morais and M.D. Willard, 2004. Electrolyte and Acid-Base Disorders. In Small Animal Clinical Diagnosis by Laboratory Methods. Eds., Willard, M.D. and H. Tvedten, 4th edn., Saunders.
40. Lacheretez, A., C. Laperrousaz, A. Kodjo, N. Brajon, D. Crevat and S. Guillossou, 2003. Diagnosis of canine parvovirus by rapid immunomigration on a membrane. Vet. Rec., 152(2): 48-50.
41. Desario, C., N. Decaro, M. Campolo, A. Cavalli, F. Cirone, G. Elia, V. Martella, E. Lorousso, M. Camero and C. Buonavoglia, 2005. Canine Parvovirus infection: which diagnostic test for virus. Journal of Virological Methods, 126: 179-185.
42. Shashidhara, Y., M. Kapil and S. Kapil, 2009. Simple tests for rapid detection of canine parvovirus antigen and antibodies. Clinical and Vaccine Immunology J., 16: 127-131.
43. Oh, J.S., G.W. Ha, Y.S. Cho, M.J. Kim, D.J. An, K.K. Hwang, Y.K. Lim, B.K. Park, B. Kang and D.S. Song, 2006. One-step immunochromatography assay kit for detecting antibodies to canine parvovirus. Clin. Vaccine Immunol., 13: 520-524.
44. Woma, T.S., 2008. The isolation and genetic characterization of canine distemper virus from domestic dogs in South Africa. M.S. Thesis, Faculty of Veterinary Science, University of Pretoria, South Africa.