Article

Cryptosporidium spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario

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Abstract — To determine the prevalence of *Cryptosporidium* spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region, Ontario, 5 of 26 clinics invited by mail survey reported their parasitological findings over 24 months. Stool samples collected by 1 clinic over 68 days were investigated for parasites by using several techniques (fecal concentration, acid-fast staining, and a *Cryptosporidium* immunoassay). The 5 clinics that provided data indicated *Toxocara* spp. as the most frequent finding. Parasitological study of 111 stool samples showed a high overall positivity rate in samples from both dogs (40%) and cats (36.6%). *Cryptosporidium* spp. antigen was detected in 7.4% and 7.3%, *Toxocara* spp. in 14.2% and 12.2%, and *Giardia* spp. 7.1% and 2.4% of dog and cat samples, respectively. The high prevalence of zoonotic parasites in the Niagara region is important, and increased awareness of their potential threat to human health is necessary. Additionally, further research into the zoonotic capacity of *Cryptosporidium* spp. and *Giardia* spp. is necessary.

Résumé — *Cryptosporidium* spp. et autres parasites entériques zoonotiques dans un échantillon de chiens et de chats domestiques de la région du Niagara en Ontario. Afin de déterminer la prévalence de *Cryptosporidium* et d'autres parasites entériques zoonotiques dans un échantillon de chiens et de chats domestiques de la région du Niagara en Ontario, 5 des 26 cliniques rejointes par un sondage postal ont rapporté leurs trouvailles parasitologiques de 24 mois et des échantillons de fèces recueillies par 1 clinique pendant 68 jours ont été analysés pour les parasites par diverses méthodes (concentration fécale, coloration acido-résistante et essai immunologique). Les 5 cliniques ayant répondu ont indiqué que *Toxocara* spp. était la trouvaille la plus fréquente. Les études parasitologiques de 111 échantillons de fèces ont révélé un taux élevé de positivité globale à la fois chez les chiens (40 %) et les chats (36,6 %). Les antigènes contre *Cryptosporidium* spp. ont été détectés chez 7,4 % et 7,3 %, *Toxocara* spp. chez 14,2 % et 12,2 % et *Giardia* spp. chez 7,1 % et 2,4 % des échantillons provenant des chiens et des chats, respectivement. La forte prévalence des parasites zoonotiques dans la région du Niagara est importante et une meilleure connaissance de leur menace potentielle à la santé humaine est nécessaire. De plus, d'autres recherches sur la capacité zoonotique de *Cryptosporidium* spp. et de *Giardia* spp. sont nécessaires.

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Introduction

Companion animals, such as dogs and cats, frequently harbor intestinal parasites that can cause human infection. Although zoonotic parasites can cause significant morbidity in all groups of the human population, they are of particular importance in vulnerable groups, such as children, the elderly, and the immunocompromised (1–3). Among a number of zoonotic parasites that infect dogs and cats, *Cryptosporidium* spp., *Giardia* spp., *Toxocara canis,* and *Toxocara cati* are of particular importance to humans (4). *Cryptosporidium* spp. can cause debilitating gastrointestinal disease that may be fatal in the immunocompromised (5). *Giardia* spp. are a frequent cause of childhood diarrhea, which, if left untreated, may become a persistent infection leading to irregular episodes of gastrointestinal illness (6). More research is needed to clarify the zoonotic potential of *Cryptosporidium* spp. and *Giardia* spp. isolates from domestic pets (7), but until solid evidence exists, care should be taken to avoid contact with potentially contaminated sources. *Toxocara canis* and *T. cati,* the common roundworms of dogs and cats, respectively, do not normally establish intestinal infections in humans, but migrating larvae can cause human toxocariasis, a systemic condition that can present as visceral larva migrans (VLM), ocular larva migrans (OLM), or both. Visceral larva migrans and OLM can seriously compromise the health of children (8,9).

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Although the importance of zoonotic parasites in dogs and cats is evident in most parts of the world, current research on zoonotic parasites remains sparse in Canada. The aim of this investigation was to conduct a pilot study to determine the frequency of *Cryptosporidium* spp. and other enteric parasites in domestic cats and dogs of the Niagara region by performing a parasitological investigation in a small sample of animals and surveying veterinary clinics in the area by mail.

Materials and methods

Mail survey

The 26 veterinary clinics listed in the telephone directory of the Niagara region in southern Ontario were contacted by mail. A data sheet requesting specific information was enclosed with the invitation letter. Clinics were asked to complete a table reporting the frequency of *Giardia* spp., *Cryptosporidium* spp., and *Toxocara* spp. in the dogs and cats attending the clinic from December 2001 to December 2003, categorized by age group, $\leq$ 6 mo and $>$ 6 mo. No information on the parasitological laboratory techniques used by the clinics was requested.

Parasitological study

One of the responding veterinary clinics with a well-established routine diagnostic laboratory wanted to know more about the study and agreed to participate in a cross-sectional investigation to determine the presence of *Cryptosporidium* spp. and other enteric parasites in a sample of its patients seen from January 22 to April 1, 2004. Feces were examined from "high risk" patients only: puppies and kittens coming for their 1st visit, older animals just adopted from shelters and rescue organizations, patients with gastrointestinal symptoms, or follow-up of recently treated patients. One stool sample per animal was analyzed for the presence of enteric parasites at both the veterinary clinic and Brock University laboratories. At the clinic, the resident laboratory technician used a sodium nitrate flotation technique (10). Briefly, 4–5 g of feces were mixed well with 35 mL of 2.8 M sodium nitrate solution (Ormond Veterinary Supply; Ancaster, Ontario). Approximately 28 mL of this solution was poured through a sieve into a glass vial. Enough sodium nitrate solution was added to the vial to form a positive meniscus and a 22- by 22-mm coverslip was then placed on top and left to stand for 10 min. The coverslip was then placed on a glass slide and systematically observed for parasites under the microscope at low (100 ×) and high dry (400 ×) magnification.

Additionally, an aliquot of approximately 2 g of fresh stool sample was placed into a glass vial containing 8 mL of 10% formalin and transferred to the laboratory at Brock University, where samples were processed by Ritchie's formalin-ethyl acetate (F/EA) concentration technique (11). Briefly, 2 mL of the formalin-fixed stool samples was transferred to 15 mL conical test tubes containing 8 mL of 10% formalin. Tubes were capped and the samples mixed well by using a vortex-stirrer. After 4 mL of ethyl acetate was added to each tube, the tubes were shaken vigorously for 30 s and then centrifuged at 1500 rpm (327 g-force) for 10 min with the cap removed. After centrifugation, the sample separated into 4 layers, from top to bottom: ethyl acetate layer, a plug of debris, 10% formalin layer, and sediment. The plug was rimmed with an applicator stick, and the top 3 layers were rapidly decanted. The sediment was stirred and a drop analyzed microscopically for parasites at 100 × and 400 ×. Additionally, samples were investigated for *Cryptosporidium* spp. by both acid-fast staining (modified cold Kinyoun [MCK]) and enzyme immunoassay, as follows: for MCK acid-fast staining (12–15), 1 portion of the sediment was smeared onto a microscope slide, air dried, fixed in absolute methanol for 3 min, stained for 10 min with the primary dye (Fuchsin-Carbol-Kynyoun 3.12%; Hartman-Leddon, Harleco, EM Science, Gibbstown, New Jersey, USA), destained for 2–3 min in acid alcohol (10% sulfuric acid in absolute ethanol), and counterstained with 3% malachite green (Harleco) for 1 min (slides were thoroughly washed with distilled water between steps). At least 50 fields of the stained slides were observed under the microscope at immersion oil magnification (1000 ×). The acid-fast staining technique was standardized with positive control samples (human samples positive for *Cryptosporidium* spp. and *Isospora belli* oocysts, and cat formalin-fixed samples positive for coccidial oocysts. The latter were treated by the same method as the unknown samples).

The enzyme immunoassay (EIA) was carried out to detect *Cryptosporidium* spp. specific antigen (CSA) on preserved (not concentrated) stool samples according to the manufacturer's instructions (ProSpecT *Cryptosporidium* Microplate Assay; Remel — Apogent Technologies, Lenexa, Kansas, USA). All samples, including controls, were tested in duplicate, and all incubations were performed at room temperature. The EIA procedure was as follows: plate wells (provided with the kit) were incubated with 200 μL of the fecal sample, which had been diluted 50% with dilution buffer, for 60 min. After shaking out the contents of the wells and washing them 3 × in the wash solution, 200 μL of enzyme conjugate was added and the plates were incubated for 30 min. The wells were then washed 5 ×, had substrate solution added, and were incubated for 10 min. Finally, 50 μL of stop solution was added to the wells, and the reaction was read visually and interpreted. Visual readings were considered negative when the reaction was colorless, indicating no or an undetectable level of *Cryptosporidium* spp. specific antigen. A reaction was considered positive when a yellow color developed; intensity of the color varied in a range from +1 to +4, as compared with a scale provided by the manufacturer.

Statistical analyses

Data were entered into an electronic database; the results were aggregated and expressed in percentages. Prevalence of different parasites was calculated as the proportion of positive specimens in the total number of investigated samples (16). Concordance of test results performed by different laboratories was calculated by using Cohen's kappa coefficient (17).

Results

Mail survey

Thirteen of the 26 clinics (50%) responded to the letter: 7 of the 13 were not able to provide data, because either they did not perform laboratory analyses or they did not keep laboratory records. Of the remaining 6 clinics, 5 completed the survey,

Table 1. Frequency of some enteric parasites in samples examined during 2001–2003, as reported in a mail survey by 5 clinics of the Niagara Region, Ontario

	Dog samples			Cat samples		
Parasite	≤ 6 months of age $n = 357$	> 6 months of age $n = 9059$	Overall prevalence $n = 9416$	≤ 6 months of age $n = 359$	> 6 months of age $n = 7801$	Overall prevalence $n = 8160$
Giardia spp.	0	6 (< 0.1%)	6 (< 0.1%)	1 (0.3%)	11 (0.1%)	12 (0.1%)
Coccidial oocysts	0	0	0	15 (4.2%)	2 (< 0.1%)	17 (0.2%)
Toxocara spp.	53 (14.8%)	20 (0.2%)	73 (0.8%)	100 (27.9%)	28 (0.4%)	128 (1.6%)
Total positive	53	26	79	116	41	158
Prevalence rate	14.8%	0.3%	0.9%	32.3%	0.53%	1.9%

Table 2. Enteric parasites detected by 2 concentration techniques[a] in 111 dog and cat stool samples collected from January to April 2004, at a veterinary clinic in the Niagara Region, Ontario

	Dog samples			Cat samples		
Parasite	≤ 6 months of age $n = 43$	> 6 months of age $n = 27$	Overall prevalence $n = 70$	≤ 6 months of age $n = 18$	> 6 months of age $n = 23$	Overall prevalence $n = 41$
Giardia spp.	5 (11.6%)	0	5 (7.1%)	0	1 (4.3%)	1 (2.4%)
Coccidial oocysts	6 (14.0%)	2 (7.4%)	8 (11.4%)	3 (16.7%)	1 (4.3%)	4 (9.7%)
Toxocara spp.	7 (16.3%)	3 (11.1%)	10 (14.2%)	4 (22.2%)	1 (4.3%)	5 (12.2%)
Taenia sp.	0	1 (3.7%)	1 (1.4%)	1 (5.6%)	1 (4.3%)	2 (4.9%)
Trichuris sp.	1 (2.3%)	1 (3.7%)	2 (2.9%)	0	0	0
Dipylidium caninum	0	0	0	1 (5.6%)	2 (8.7%)	3 (7.3%)
Hookworms	2 (4.7%)	0	2 (2.9%)	0	0	0
Total positive	21	7	28	8	7	15
Prevalence rate	48.8%	25.9%	40%	44.4%	30.4%	36.6%

[a] Aggregated results of sodium nitrate and formalin-ether concentration techniques, 95% agreement

Table 3. Details of 8 positive samples for *Crypstosporidium* specific antigen detected by an enzyme immunoassay[a] in 109 dog and cat stool samples collected between January and April 2004

Subject number and species	Age of animal	Microscopy after concentration[b]	Modified acid-fast staining	Enzyme immunoassay[a]
1, dog	10 weeks	*Giardia* sp.	Negative	Positive +
2, dog	7 weeks	Negative	Negative	Positive +
3, dog	11 weeks	Negative	Negative	Positive +
4, dog	9 weeks	Negative	Negative	Positive +
5, dog	3 months	*Toxocara* sp.	Negative	Positive +++
6, cat	6 months	*Toxocara* sp.	Negative	Positive +
7, cat	6 months	Negative	Negative	Positive ++
8, cat	7 months	Negative	Negative	Positive +++

[a] ProSpecT (*Cryptosporidium* Microplate Assay, Remel — Apogent Technologies Inc.)
[b] Aggregated results of sodium nitrate and formalin-ether concentration techniques, 95% agreement

and 1 requested more information. A summary of the data provided by the 5 responding clinics can be seen in Table 1. These data indicated a low overall rate of parasite infection, with the majority of intestinal infections occurring in dogs and cats ≤ 6 mo of age, in which positivity rates were 14.8% and 32.3%, respectively. In this age group, 100% of dog and 88% of cat samples reported as positive contained *Toxocara* spp. eggs. Since special staining or immunoassays specific for *Cryptosporidium* spp. were not part of the routine examinations performed by the clinics, none of them provided data for this parasite. The clinic that requested more information agreed to participate in a parasitological study.

Parasitological study

In total, 111 samples, 70 single samples from dogs (aged 2 mo to 14 y) and 41 single samples from cats (aged 1.5 mo to 8 y) were examined by both concentration techniques (sodium nitrate and F/EA), as shown in Table 2. When comparing results from both techniques, discrepancies were observed in only 5 samples in which coccidial oocysts were reported by the Brock University laboratory only (concordance 95%, $P < 0.0001$). Overall, a total of 7 species or groups of parasites were identified microscopically. Out of the 70 dog samples, 42 (60%) were negative and 28 (40%) were positive, of which 23 (82%) had a single species-infection and 5 (18%) presented mixed infections. Out of 41 cat samples, 26 (63.4%) were negative and 15 (36.6%) were positive, of which 12 (80%) had a single species-infection and 3 (20%) presented mixed infections. As seen in Table 2, younger animals were more likely to be infected than older ones. Among the parasites identified, *Toxocara* spp. were the most frequent, for a detected prevalence of 14.2% and 12.2% in the canine and feline stool samples, respectively. *Giardia* spp. were identified in 7% of dogs (all ≤ 6 mo of age) and in 2.4% (1 animal) of cat samples. Other parasites identified are shown in Table 2.

Crypstosporidium specific antigen detection

One-hundred and nine samples (68 dog, 41 cat) were tested for *Cryptosporidium* spp. specific antigen. None of the samples was positive for oocysts with the acid-fast staining technique. In contrast, 8 samples (5 dogs and 3 cats) were positive for the specific antigen when tested by the immunoassay. The EIA results were 100% reproducible, with all pairs of samples having identical results, either positive or negative. As shown in Table 3, *Cryptosporidium* spp. specific antigen was detected in 7.4% (5/68) of dog samples and 7.3% (3/41) cat samples. All positive samples belonged to animals ≤ 7 mo of age. More details of positive animals are shown in Table 3.

Discussion

Few investigations have examined the prevalence of *Cryptosporidium* spp., *Toxocara* spp., and other parasitic infections in dogs and cats in Canada. With the current changing patterns in infectious disease distribution and occurrence (18–21), surveillance of zoonotic diseases in companion animals is necessary. The present study was designed as a pilot research project for 1 of the team members (RS) as part of his academic curriculum at Brock University. This restricted the duration of the investigation to the period indicated above.

As shown in the results, the mail survey had a low response rate, indicating that our survey failed to generate enough interest in the target clinics. Perhaps having provided a better incentive than just sharing their data would have been beneficial and should be considered for further investigations. From the data provided, potential differences in laboratory techniques used by the different clinics made it difficult to compare results and draw conclusions. Nevertheless, the data obtained showed that helminth infections were the most reported, with *Toxocara* spp. the most frequently diagnosed in both dogs and cats.

The 111 investigated samples demonstrated a high prevalence of enteric parasites, with an overall positivity rate of 40% for dogs and 36.6% for cats, and with higher rates in animals ≤ 6 mo of age. Consistent with the data obtained from the mail survey, the most frequently detected species in all fecal samples examined was *Toxocara,* with a total prevalence of 14.2% for dogs and 12.2% for cats. The relative ease of identifying *Toxocara* spp. ova and the high probability of detecting patent infections (a female worm can produce 100 000 eggs/d) (22) may be the reason why *Toxocara* spp. are the most frequently detected helminth endoparasites in the said animals. Of the 10 dogs found infected with *T. canis,* 7 were ≤ 6 mo of age (range: 2 mo to 8 y) and 3 were older (2 were 2 y old and 1 was 8 y old). Of the 5 cats testing positive for *T. cati,* all but 1 were ≤ 6 mo of age (range: 3 mo to 3 y of age). These results show that even though patent toxocariasis is more frequent in the young (22), animals of all ages may be passing eggs to the environment. The paucity of similar recent published work in Canadian companion animals prevents comparison, but the prevalence of *Toxocara* spp. found in the present study was considerably higher than that observed between 1999 and 2001 at the Animal Health Laboratory, University of Guelph, in which 3% of dog and 9% of cat samples were positive for *T. canis* and *T. cati* eggs, respectively (23). The most plausible explanation for the high prevalence found in the present study is that the samples examined belonged to high risk animals, as opposed to the entire population attending the clinic. For the participating clinic, it is standard practice to examine all animals presented for the 1st visit, regardless of their age, for enteric parasites. In most cases, this includes very young animals; older individuals, when recently adopted; or animals with gastrointestinal symptoms. Parasitized animals are given specific treatment and follow-up fecal examination. The quality of care provided by this clinic is ideal, and although not all veterinary clinics have laboratory facilities, all efforts should made to achieve early and proper diagnosis, as well as specific treatment and follow-up. This practice results in the twofold benefit of keeping animals healthy and preventing environmental contamination. Our findings also highlight the significance of city by-laws that require owners to pick up after their pets and the need for owners to be educated about these issues. It would be very interesting to conduct further research to determine the rate of infection in animals from shelters and rescue facilities, as well as in stray animals. Even though in the Niagara region stray dogs are seldom observed, cats are frequently seen wandering around neighbourhoods and being potential spreaders of zoonotic parasites.

The importance of *Toxocara* spp. as a cause of human infections has been described extensively, but their current prevalence in Canada remains unknown (23). Several cases of VLM led to an investigation of *Toxocara* spp. in stray dogs and cats in Halifax in 1971–1972 (24), but a report published 1981 concluded that VLM posed little risk to the health of children in the Toronto area (25). However, if VLM is not considered among the differential diagnoses in children presenting with suggestive symptoms, many cases may go undiagnosed, leading to underreporting of the condition.

Another group of important parasites identified in both dogs and cats in the present study was hookworms; as in the other parasite infections, animals ≤ 6 mo of age were more likely to be infected. Two species of these small roundworms, *Ancylostoma braziliensis* and *A. caninum,* can cause human disease in the form of cutaneous larva migrans (CLM), especially in young children (23). Locally acquired cases of CLM have not been reported in the Canadian literature, which probably reflects a true absence of cases, since *Uncinaria stenocephala,* the most common hookworm of dogs in Canada (26), is not associated with the condition. The prevalence of *Giardia* spp. found in the present investigation (7.1% in dogs and 2.3% in cats) is similar to that of other findings in Canada and the United States (27). On the other hand, the low report of *G. lamblia* by the clinics participating in the mail survey, most likely indicates the use of low-sensitivity parasitologic techniques in their laboratories rather than a decrease in the prevalence of this ubiquitous parasite. *Giardia lamblia* is the most frequently reported human parasite in Canada (28) and, albeit controversial (7), the role of domestic pets as a source of infection should not be overlooked. For this investigation, only morphological diagnosis was made and the possibility of zoonotic transmission from these pets to humans remains an open question. Further studies aiming to demonstrate zoonotic potential should include characterization

of the parasite into the 8 major assembleges, which apparently have distinct host preferences (7).

In the present study, the acid-fast staining technique was negative for oocysts of *Cryptosporidium* spp., although the specific antigen was detected by the EIA in 8 samples. Other studies report a high concordance between both techniques, but the superior sensitivity of immunologic techniques over microscopic examination is widely recognized (29–31). The discrepancy between microscopic examination and immunoassay in the present study was most likely due to a low number of oocysts present in the samples (31). Nevertheless, the positivity for *Cryptosporidium* spp. found with the EIA is significant and warrants further investigation. *Cryptosporidium* spp. infection has been extensively reported in cattle in Canada (32), but to our knowledge, their presence in companion cats and dogs in Canada has not been published. More studies should be undertaken to confirm the presence of this apicomplexa parasite in local domestic dogs and cats. Furthermore, molecular studies should be used to determine the distinct genotypes involved. Similar to *Giardia* spp., different *Cryptosporidium* spp. genotypes display different host specificity. It appears that, so far, the genotype *C. hominis,* usually infecting humans, and the genotype *C. parvum,* usually infecting cattle, are the most prevalent in humans (7), but a few cases of *Crypstosporidium felis* infection have been reported to cause illness not only in immunocompromised humans (33,34) but also in immunocompetent hosts (34). Moreover, *Cryptosporidium* "dog-type" has been identified as cause of illness in humans in the United Kingdom (34). Furthermore, as shown in Table 3, all *Cryptosporidium*-positive animals were very young, underlining the importance of household hygienic measures to avoid fecal contamination. As *Cryptosporidium* spp. oocysts shed in feces are immediately infective (7), if any of these isolates were able to infect humans, cases of serious illness could occur, especially in immunocompromised people.

As a result of climate change, human travel, and global commerce, new infectious diseases are emerging in Canada and zoonotic infections from domestic pets may start emerging in the near future. Nowadays, veterinary practices have the important responsibility of educating pet owners about the potential risk of zoonotic parasites and the different measures that can be taken for their control and prevention (22). Moreover, veterinary clinics could play an important role in tracking trends of parasite infections that may have public health repercussions.

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OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases

Various Authors. World Organisation for Animal Health (OIE), Paris, France, 2002. ISBN 92-9044-575-0. 50 €.

This book, together with *ISO/IEC 17025:1999, General requirements for the competence of testing and calibration laboratories,* which it interprets, and the *OIE Manual of Standards for Diagnostic Tests and Vaccines,* provide information useful for laboratories conducting tests to enable the international movement of animals.

The extensive glossary, with precise definitions of assay parameters, will ease communication between scientists working in the laboratories of various countries, who may be accustomed to different terminology.

The OIE Standard for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Diseases follows the format familiar to laboratory personnel who conform to ISO/IEC 17025. It describes the management and technical requirements, including items such as document control, corrective actions, records, internal audits, personnel, equipment and reports, to name a few. Workers in veterinary laboratories who have been audited to ISO/IEC 17025:1999 or expect to be audited to ISO/IEC 17025:2005 will appreciate the further clarification of the requirements, as they pertain to veterinary laboratories, for items such as test methods. In ISO/IEC 17025, the emphasis is on selection of appropriate test methods to meet the client's needs. In this standard for veterinary laboratories, the emphasis is on the selection of appropriate test methods that are widely accepted by scientists and regulators. Client agreement is required, of course, and testing laboratories must inform the client of the test method chosen and the reasons for the choice.

OIE Guide 1: Validation of Diagnostic Assays for Infectious Diseases describes validation as a multi-stage process. Scientists can use this information to decide how far to proceed with validation, based on a new test's performance at each stage. Validation of test methods is described in the context of animal disease diagnostics and the calculation of various estimates of assay performance is explained.

OIE Guide 2: International Reference Standards for Antibody Assays describes the preparation, approval, and use of standards. Essential for standardizing tests within and between laboratories, these reference standards are also useful for verifying that tests are performed correctly and for trouble-shooting problems that may occur while conducting tests.

OIE Guide 3: Laboratory Proficiency Testing provides guidance for the evaluation of veterinary laboratories. This is key to fostering trust in the test results reported by a laboratory or country, when importing or exporting animals. Proficiency testing may be used to evaluate a technician, a test method, or a laboratory.

OIE Guides 1, 2, and 3 provide invaluable information for the harmonization of testing between laboratories, which is crucial for those carrying out tests for international movement of animals.

Laboratories that are already certified to ISO/IEC 17025 will have arrived at a workable interpretation of the ISO standard on their own and may find that this publication does not offer much additional information. This book will be particularly useful to veterinary laboratories that wish to implement a quality system and obtain ISO certification. Veterinarians collecting specimens to submit to such laboratories will benefit from reading this publication.

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