THESIS

ACURRACY ASSESSMENT OF FOUR DIAGNOSTIC TESTS FOR THE DETECTION OF *GIARDIA* AND *CRYPTOSPORIDIUM* IN THE ABSENCE OF GOLD STANDARD: A BAYESIAN APPROACH

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ABSTRACT

ACURRACY ASSESMENT OF FOUR DIAGNOSTIC TESTS FOR THE DETECTION OF GIARDIA AND CRYPTOSPORIDIUM IN THE ABSENCE OF GOLD STANDARD: A BAYESIAN APPROACH

Giardia and *Cryptosporidium* are important parasites that cause gastrointestinal disease in numerous animal species including dogs and cats. The accurate diagnostic of this diseases is cucial for the aplication of preventive measures and precise treatment. Estimation of test accuraccy is not difficult when a reference test (gold standard) is available. However, when a gold standard test is not available the Bayesian Latent Class (BLC) Analysis is an effective analytical tool for the estimation of diagnostic accuracy. The aim of this study was to estimate the sensitivity (Se) and specificity (Sp) of four commercial diagnostic kits using BLC. The four diagnostic tests were (1) Merifluor®Direct Fluorecence Antigen (DFA; *Giardia* /*Cryptosporidium*; Meridian Diagnostics, Inc., Cincinnati, Ohio), (2) IVD®DFA (*Giardia* /*Cryptosporidium*; IDV Research Inc., Carlsbad, CA), (3) IVD Microwell ELISA® (*Giardia* ; IDV Research Inc., Carlsbad, CA), (4) and IDEXX SNAP® (*Giardia* ; IDEXX Laboratories Inc., Westbrook, ME). The results from 201 laboratory analysed samples, the prior distributions elicited from three experts, and the consistency of samples as splitting covariate were used as inputs for the BCL models. The estimated Se and Sp of the tests were 87.7% and 97.3% (Merifluor-*Cryptosporidium*), 68.0% and 99.1% (IVD-*Cryptosporidium*), 93.6% and 97.9% (Merifluor-*Giardia*), 96.1% and 97.9% (IVD-*Giardia*), 86.0% and 98.2% (ELISA-*Giardia*), and 84.8% and 98.0% (SNAP-*Giardia*) respectively. The prevalence for non-diarrheic versus

diarrheic samples were 2.3% and 4.8% (*Cryptosporidium*), and 6.9% and 13.5% (*Giardia*) respectively. We were able to use BLC to assess the sensitivity and specificity of the four commercial diagnostic tests. We ran 36 models and used objective indicators of the per formances of the models to choose the best model for estimation of parameters. The results of the study indicated that Merifluor, IVD, and ELISA are equally suitable as diagnostic tests for detection of *Giardia*. For detection of *Cryptosporidium*, Merifluor was more accurate than the IVD test.

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1 LITERATURE REVIEW

1.1 GIARDIASIS IN CATS AND DOGS

1.1.1 Etiology

Giardia duodenalis (syn. *intestinalis, lamblia*) is a primitive eukaryotic species of the Phylum Metamonada and order *Giardia* (Pluzer, Ongerth, & Karanis, 2010; Cavalier-Smith, 2003). The following is the taxonomic classification of the genus *Giardia* according to the systematic taxonomy based on genetic, structural, and biochemical data:

Kingdom	Eukaryote
Phylum	Metamonada
Subphylum	Trichozoa – flagellated protozoans
Superclass	Eopharyngia
Class	Trepomonadea
Subclass	Diplozoa
Order	Giardiida
Family	Giardiae
Genus	*Giardia* Cavalier-Smith, 2003 (Pluzer, Ongerth, & Karanis, 2010)

The organisms of the genus *Giardia* are a very unusual kind of ancient eukaryotes as they share many characteristics with anaerobic prokaryotes. *Giardia* does not have the common intracellular organelles such as mitochondria, peroxisomes, or even a traditional Golgi apparatus that characterizes most of eukaryotes (Pluzer, Ongerth, & Karanis, 2010; Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010). However, during encystation, large secretory compartments are developed; these compartments show several biochemical characteristics of the

Golgi cisternae, this pseudo-organelles contain the essential compound for the cyst wall development (Pluzer, Ongerth, & Karanis, 2010; Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010).

In the past, the light microscopy was the most common tool for differentiating species of microorganisms. Then, the use of electro-microscopy increases the amount of morphologic information available for species identification. Six species of *Giardia* have been identified based on morphologic characteristics as feature of ventrolateral flange, marginal groove, ventral disc, and flagellum (Pluzer, Ongerth, & Karanis, 2010). Five from the six species were isolated from amphibians (*G. agilis*), birds (*G. ardeae, G. psittaci*), mice (*G. muris*), and voles (*G. microti*). The sixth species included *Giardia* strains isolated from large range of others mammalian hosts. These strains share several morphological features and were named as *G. duodelanlis* (Pluzer, Ongerth, & Karanis, 2010). Later on, with the use of modern molecular techniques such as RNA gene sequencing, all species have been defined (Pluzer, Ongerth, & Karanis, 2010).

The stains of *Giardia* derived from human isolates were earlier assigned to a separate species (*G. lamblia*) and the major lineages defined on these human-derived isolates were designated as assemblages A and B (Pluzer, Ongerth, & Karanis, 2010). *Giardia duodenalis*, derived from animal isolates, shows a similar genetic spectrum. Some isolates appear to be identical to genotypes found in humans, while others represent genotypes that are apparently host specific (Pluzer, Ongerth, & Karanis, 2010). These findings are relevant when the possibility of giardiasis as a zoonosis is taking in to account (see **1.1.10** section below).

The different assemblages of *G. duodenalis* have been assigned after finding substantial sequence differences in the genes, such as the glutamate dehydrogenase/gdh, triosephosphate isomerase/tpi, and β-giardin/bg genes (Pluzer, Ongerth, & Karanis, 2010). Assemblages A to G

have been defined by molecular techniques within the *G. duodenalis* morphological group. It has been determined that dogs are primarily infected by assemblages C and D, whereas cats are primarily infected by assemblage F. Assemblages A and B have also been identified in feces from dogs and cats by DNA amplification (Pluzer, Ongerth, & Karanis, 2010; Scorza & Lappin, 2012).

1.1.2 Morphology

Giardia has two main life forms: trophozoite and cyst

The trophozoite (Figure 1), which is the active and motile form that habits the lumen of the intestinal tract, is approximately 15 µm long, 8 µm wide, and 3 µm thick (Kirkpatrik, 1987). One of the most relevant trophozoite morphologic characteristic is its drop shape and the organization of its organelles: two nuclei, the axomeres, and the median bodies, which resemble a smiley, face (Scorza & Lappin, 2012).

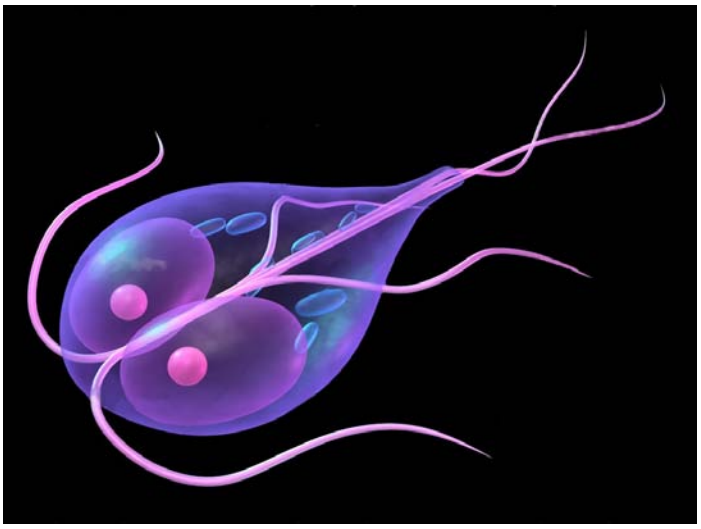


Figure 1. Scheme of a *Giardia* trophozoite anatomy (Google Image search; http://www.vetlive.com/2011/07/12/*Giardia* -in-dogs/).

The protozoans of this order are flagellates with a flattened ventral face occupied by an adhesive disk, which attaches the parasite to the intestinal mucosa of its host (Figure 2). Some of the

organelles may be visible in light microscopy preparations, such as four pair of flagella, two nuclei, the axomeres, and the median bodies (Kirkpatrik, 1987). The cell tapers posteriorly where the two caudal flagella rise; all flagella are directed posteriorly. The trophozoite adheres on the brush border of the intestinal epithelial cells and the sucking force is generated by the beating of the ventral enlarged flagella (Scorza & Lappin, 2012).

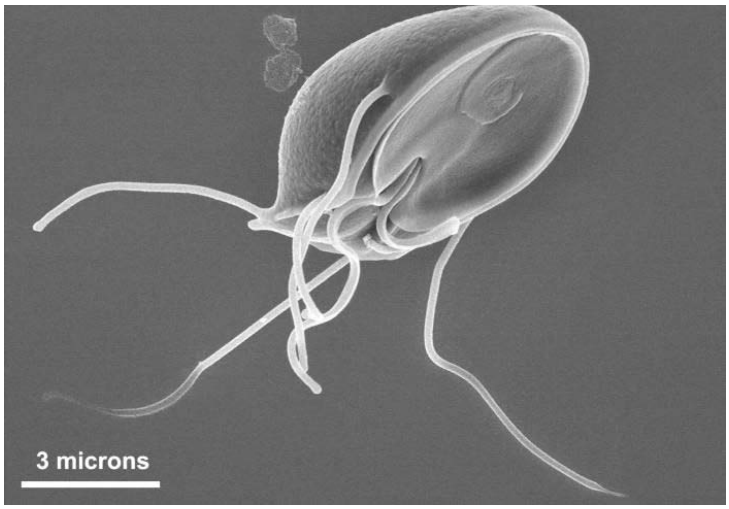


Figure 2. This scanning electron micrograph (SEM) clearly shows the ventral surface of a *Giardia muris* trophozoite. The adhesive disk facilitates adherence of the protozoan to the intestinal surface. Created: 2000 (Public Health Image Library Photographer: Dr. Stan Erlandsen).

The cyst, which is the environmental resistant stage of the parasite, has an oval or ellipsoidal form with approximately 12 µm long and 7 µm wide. This cyst contains two incompletely separated trophozoites. This stage is resistant to some environmental conditions and can last several months in wet and cold conditions (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010; Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010). This stage is the most common form of the parasite used for diagnostic, and most of the diagnostic tests are designed to detect or identify some of the cyst wall components.

1.1.3 Life cycle

After ingestion of the cyst, it becomes metabolically active. The excystation process takes approximately 15 minutes. The gastric acid and pancreatic enzymes trigger the excystation process on the duodenum. The liberated excyzoite undergoes cytokinesis separating the trophozoites (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010).

After a short, not fully understood, biochemical mediated maturation process, the two released trophozoites attach to the brush border of the villous epithelium by its ventral discs (specific characteristic of the genus *Giardia)* (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010).

The trophozoites multiply by binary fission and encyst in the intestinal tract. The mechanisms of encystation have yet been described completely. The encystation process is an induced response triggered by several host factors such as high levels of bile, low levels of cholesterol, and increase in the pH (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010). The first step that takes place for encystation is the internalization of the flagella. Additionally to this, the fragmentation of the ventral disk favors the loss of ability to attach to the intestinal wall. The parasite gradually rounds up and decreases its metabolism to enter in a stage of dormancy. Finally, the encystation specific vesicles selectively transport the cyst wall proteins to the surface and form the cyst wall. Before encystation, the trophozoite starts a division cycle that ends after excystation. This division produces two nuclei pairs that are observable in the formed cyst (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010).

1.1.4 Pathogenesis

The pathophysiological mechanisms underlying symptomatic giardiasis remain incompletely understood. However, it is widely accepted that the clinical symptoms of giardiasis arise due to a

combination of both host and parasitic factors. One of the main clinical signs of giardiasis is the diarrhea, which appears to be caused by a combination of malabsorption and hypersecretion (Scorza & Lappin, 2012; Cotton, Beatty, & Buret, 2011). The common clinical sings found in patients with giardiasis are often related to four main pathological events: i) the increase of apoptosis of epithelial cells, ii) the increase of intestinal permeability, iii) the disruption of cellular apical junctions, and iv) the shortening of the brush border microvilli (Figure 3) (Cotton, Beatty, & Buret, 2011).

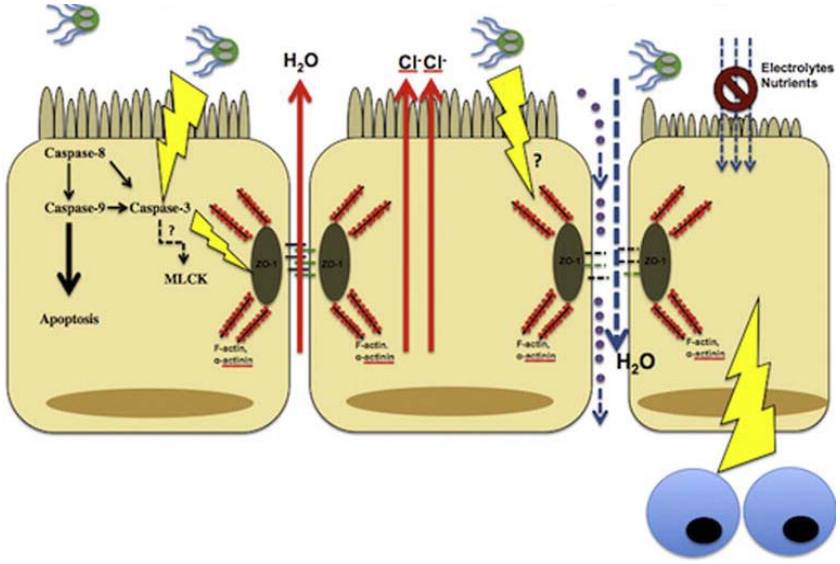


Figure 3. The pathophysiological manifestations of giardiasis (Elsevier Licensed 3317710976907)

The increase of epithelial cell apoptosis rates could occur via activation of Caspases-9 and 3. However, the precise mechanisms are still unknown. Some other parasitic factors may activate hypersecretion of chloride, which may contribute to diarrhea (Cotton, Beatty, & Buret, 2011). *Giardia* also increases the intestinal permeability during giardiasis by disrupting apical junctional complex components (including F-actin, ZO-1, claudin-1, and α-actinin) in a Caspase-3 dependent manner. The increase of the epithelial permeability is due, at least in part, to the

activation of Myosin Light Chain Kinase (MLCK). The lack of impermeability allows the translocation of antigens into the subepithelial tissue (Cotton, Beatty, & Buret, 2011).

Another important event is the shortening of the brush border microvilli; an effect mediated by host CD8+ T lymphocytes. Consequently, the absorptive surface area is reduced during giardiasis, resulting in digestive deficiencies and contributing to maldigestion; signs commonly associated with giardiasis. Additionally, the microvillus lesion is unable to absorb glucose and electrolytes effectively, resulting in the decrease of water uptake and subsequent malabsorptive diarrhea (Cotton, Beatty, & Buret, 2011).

1.1.5 Epidemiology

Giardia infects several mammalian species worldwide including humans. Many studies have established the prevalence of *Giardia* in dogs and cats (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010; Mohamed, Glickman, Jr., Lund, & Moore, 2013). However, results tend to vary considerably because of the difference in the tests used, and the differences in population and region where the study was done (Thomson, Palmer, & O'Handley, 2008; Ballweber, Xiao, Bowman, Kahn, & Cama, 2010).

The affected patients acquire the environmental resistant *Giardia* cysts by oral ingestion -- commonly from contaminated food or water-- or by grooming when the coat is contaminated with feces (Mohamed, Glickman, Jr., Lund, & Moore, 2013). Carnivorism is another possible way of acquiring *Giardia*, if the organism is present in the prey intestine (Kirkpatrik, 1987).

The prepatent period of giardiasis ranges from 6 to 16 days in cats and from 4 to 12 days in dogs (Payne & Artzer, 2009). The number of cysts shed by an infected patient varies considerably, ranging from undetectable amounts to thousands of cysts per gram of feces. The peaks of cyst shedding occur sporadically, presenting shedding peaks every 2 to 7 days (Kirkpatrick & Farrell,

1984). In a recent large-scale study (Mohamed, Glickman, Jr., Lund, & Moore, 2013), living in crowded and unsanitary conditions was identified as an important risk; factor for *Giardia* infection in dogs. In addition, young puppies and intact individuals have more are on higher risk of having the disease than when compared to other populations (Mohamed, Glickman, Jr., Lund, & Moore, 2013) . An identified risk factor of giardiasis is to live in places that favors the environmental conditions that allow the cysts to survive longer, thereof, favoring higher contact and contagion rates (Mohamed, Glickman, Jr., Lund, & Moore, 2013). In other studies, only age and living in community were significant risk factors (Yang, et al., 2014; Bajer, Bednarska, & Rodo, 2011; Mark-Carew, et al., 2013).

1.1.6 Clinical Findings

Most of infected cats and dogs with *Giardia* do not show any clinical manifestation of the disease. However, some patients may present with serious illness (Thomson, Palmer, & O'Handley, 2008; Payne & Artzer, 2009). The clinical signs may occur continuously or intermittently, or they may disappear after initiate treatment with nonspecific antidiarrheics (Rossignol, 2010; Thomson, Palmer, & O'Handley, 2008). The clinical signs can range from slight abdominal discomfort to severe abdominal pain (Payne & Artzer, 2009). Predominant signs of giardiasis include those expected from maldigestion and malabsorption of nutrients: pale and malodorous feces, steatorrhea, chronic diarrhea, and weight loss or poor weight gain despite normal appetite (Thomson, Palmer, & O'Handley, 2008; Kirkpatrik, 1987; Payne & Artzer, 2009).

Since *Giardia* is not usually entero-invasive, very watery or hemorrhagic diarrhea is rare; it may occur if co-infection with other pathogens can occur organisms present (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010). Most affected cats and dogs are not-febrile, do not

vomit, and have serum total protein concentration and complete blood counts values within reference limits (Thomson, Palmer, & O'Handley, 2008; Payne & Artzer, 2009).

1.1.7 Diagnosis

There are numerous tests for the diagnosis of *Giardia* in dogs and cats. These tests range from the most conventional fecal microscopic examination (ME) to the modern quantitative Polymerase Chain Reaction (q-PCR) used for the identification of genetic markers (Tangtrongsup & Scorza, 2010; Koehler, Jex, Haydon, Stevens, & Gasser, 2013).

The most serious problem of diagnostic tests identifying *Giardia* is that none of them is sensitive enough to detect all the true positive cases when just one sample is examined. The combination of tests or the examination of interval samples is an option to increase sensitivity. However, this kind of process increases the medical costs by at least twice than using a more sensitive tool (Tangtrongsup & Scorza, 2010). Below, we describe the common diagnostic techniques for the detection of *Giardia*.

1.1.7.1 Conventional Microscopy

In some cases, with patients that have very watery diarrhea and hypermotility, some trophozoites may be found in the fresh fecal samples immediately examined after collection (100x for motility and 400x for morphologic details; light microscopy). This procedure uses a small quantity of the diarrheic sample or mucus mixed with warm (37°C) NaCl normal saline solution and covering with a cover-slip (Koehler, Jex, Haydon, Stevens, & Gasser, 2013; Tangtrongsup & Scorza, 2010). This is a highly specific detection tool but not very sensitive, since the detection of trophozoites of *Giardia* is confirmatory of its presence, but not finding trophozoites does not indicate its absence. Further tests need to be done to confirm a negative sample (Goka, Rolston, Mathan, & Farthing, 1990). The trophozoites are motile for a few hours and lose their motility at

room temperatures. The motility pattern allows the examiner to differentiate *Giardia* trophozoites from trichomonads that are similar in size. The trichomonads can be differentiated by the presence of the undulating membrane, the rolling form of motility, the lack of concave surface, and the presence of a single nucleus. The use of stains such as iodine and iron-haematoxylin, giemsa, or trichrome may enhance the ability to identify cellular structures of the trophozoites (Koehler, Jex, Haydon, Stevens, & Gasser, 2013). If the microscopic examination is inconclusive, detection of fecal antigen can be used for confirmation. In addition, nucleic acids amplification may be used whether for identification of species or identification of specific genetic markers is a matter of interest (Tangtrongsup & Scorza, 2010).

The most of the available diagnostic tools for the detection of *Giardia* are based on the detection and/or identification of the cysts in the fecal specimens. Concentration techniques have been routinely used in order to increase the detection rates over wet mounts. In the same manner, staining procedures have been incorporated to the laboratory protocols in order to decrease misdiagnosis (Tangtrongsup & Scorza, 2010). One of the most common procedures for identification of *Giardia* cysts is staining procedure with Lugol's iodine after centrifugation/flotation in Zinc-Sulfate media (Koehler, Jex, Haydon, Stevens, & Gasser, 2013; Tangtrongsup & Scorza, 2010). Sensitivity of Zinc-Sulfate has been repoted as low as 45% in one sample (Rishniw, Liotta, Bellosa, Bowman, & Simpson, 2010). Then, it is recommended to examine at least three fecal samples from every other day, to increase the probability of detecting a true positive sample (Berghoff & Steiner, 2011), However, this imply that the time of delivering laboratory results would increase and the practicality of the diagnostic may be a matter of concern if we take the willingness of the clients to pursue this procedure.

The duodenal aspirate examination has been considered the most sensitive test for detection of *Giardia.* This technique requires general anesthesia, special endoscopic or surgical equipment, more complex expertise, and immediate examination of the sediment of duodenal content in warm slides (37°C), looking for motile trophozoites. However, this test is not widely used because its complexity and because it is invasive compared to other techniques invasiveness (Goka, Rolston, Mathan, & Farthing, 1990; Koehler, Jex, Haydon, Stevens, & Gasser, 2013).

1.1.7.2 Immunochemical antigen detection

Direct immuno-fluorescent antigen (DFA) detection tests are one of the most common techniques for the detection of *Giardia* cysts in fecal samples; considered as the reference tests by some researchers (Aziz, Beck, Lux, & Hudson, 2001; Garcia & Shimizu, 1997; Johnston, Ballard, Beach, Causer, & Wilkins, 2003). This technique uses fluorescein-labeled monoclonal antibodies to target cyst wall proteins (Koehler, Jex, Haydon, Stevens, & Gasser, 2013; Tangtrongsup & Scorza, 2010). This test has low rate of false positives (high specificity), which is one of its more significant features as diagnostic tool. Its high specificity is due to both, the specific target of the monoclonal antibodies and the morphology recognition of the cysts by the technician (Koehler, Jex, Haydon, Stevens, & Gasser, 2013; Rishniw, Liotta, Bellosa, Bowman, & Simpson, 2010; Johnston, Ballard, Beach, Causer, & Wilkins, 2003; Aziz, Beck, Lux, & Hudson, 2001; Garcia & Shimizu, 1997). Another important feature of this diagnostic test is that the available commercial kits detect *Cryptosporidium* spp. as well. This is a relevant feature since *Cryptosporidium* and *Giardia* are frequently found as confections and both can be associated with small intestine pathology (Thomson, Palmer, & O'Handley, 2008). One of the disadvantages of this technique is that requires the use of a fluorescent microscope, which is not

usually available in common practices. This feature needs to be taken into account when deciding what test is more suitable for a particular situation.

Another popular technique is the enzyme linked immunosorbent assay (ELISA). Some of these assays are commercialized as point-of-care testing (POCT) kits due to their practicality and rapidity, and because they do not require specific training and, in most of the cases, they do not require complex equipments (Scorza & Lappin, 2012; Tangtrongsup & Scorza, 2010). One of the main concerns of these tests is the inconsistency of the results in different populations, showing different sensitivity values. Some of these discrepancies could be explained by the use of different reference tests to calculate the values (Zimmerman & Needham, 1995; Johnston, Ballard, Beach, Causer, & Wilkins, 2003; Aziz, Beck, Lux, & Hudson, 2001; Garcia & Shimizu, 1997; Mekaru, Marks, Felley, Chouicha, & Kass, 2007).

Since none of those tests has enough sensitivity to confirm the infection with *Giardia*, the Companion Animal Parasite Control (CAPC www.capcvet.org) recommends testing the suspicious fecal samples from dogs and cats with a combination of direct smear, fecal centrifugation flotation, and any antigen detection test. In addition, it is recommended to perform tests throughout several days to increase the probability of finding the cysts (Tangtrongsup & Scorza, 2010; Strand, Robertson, Hanevik, Alvsva, & Langeland, 2008).

1.1.7.3 Molecular Techniques

The use of molecular techniques has not been extensively used for regular diagnosis of *Giardia*. However, the molecular techniques have played a crucial role in the research and understanding of the biology, epidemiology, ecology, and population genetics of the genus *Giardia* (Ankarklev, Jerlstrom-Hultqvist, Ringqvist, Troell, & Svard, 2010; Feng & Xiao, 2011; Thomson R. , 2004). Most of the available techniques rely on the specific amplification of one or more loci in small

amounts of samples (Koehler, Jex, Haydon, Stevens, & Gasser, 2013). PCR-based methods are common molecular tools used for the identification and research of *Giardia* assemblages (Koehler, Jex, Haydon, Stevens, & Gasser, 2013). The isolation of nucleic acids is crucial for the effective utilization of PCR-based methods. Some of the methods that have been assessed include sonication, freeze/thaw cycling, phenol clorophormchloroform, among others (Adamska, Leońska-Duniec, Maciejewska, Sawczuk, & Skotarczak, 2010; Babaei, Oormazdi, Rezaie, Rezaeian, & Razmjou, 2011; Koehler, Jex, Haydon, Stevens, & Gasser, 2013). Gene markers beta-giardine (*bg*), triose-phosphate isomerase, and glutamate dehydrogenase, have been studied with the small subunit (SSU) of the nuclear ribosomal RNA (rRNA) gene to provide the basis for the molecular research of *Giardia* (Feng & Xiao, 2012).

Random amplification of polymorphic DNA analysis (RAPD) had been used because of its ability to amplify small amounts of DNA and its capability to rapidly screen for variation without requiring previous sequencing (Deng & Cliver, 1999; Pelayo, Fraga, Núñez, Mendoza, Torres, & Finlay, 2003). However, this technique presents significant problems of specificity and reproducibility, due to the stringency variability of the genomic material (MacPherson, Eckstein, Scoles, & Gajadhar, 1993). Restriction fragment length polymorphism (RFLP), specific PCR and sequencing are the most common tools for identification and classification of *Giardia* (Caccio, Beck, Almeida, Bajer, & Pozio, 2010; Feng & Xiao, 2011; Thompson & P.T. Monis, 2004). RFLP has demonstrated to be useful for classification and research of *Giardia*. However, some of its limitations are that not all restriction enzymes detect all variations in a marker (Koehler, Jex, Haydon, Stevens, & Gasser, 2013). The gold standard for recognition of gene variations is the sequence-based analysis. This tool allow for comparisons within and among populations with the benefit of being suitable for the construction of phylogenetic trees (Caccio, Beck, Almeida,

Bajer, & Pozio, 2010). Real time PCR is a molecular tool that not only allows for specific identification of assemblages and subassemblies but also allows for quantification of the concentration of organisms in the samples (José L. Alonso, 2011; Guy, Payment, Krull, & Horgen, 2003). Novel molecular tools are often being designed or refined according to overcome technical and logistical limitations. In addition, the increase of computational analysis tools broadens the scope of the molecular tools usage to better understand the biology of *Giardia.*

1.1.8 Treatment

In practice, the treatments for *Giardia* are based on those used for humans (see Table 1) (Tangtrongsup & Scorza, 2010; Gardner & Hill, 2001). The first goal for the treatment of giardiasis is to stop the diarrhea; a secondary goal should be the elimination of the parasite, which is important when the assemblage found has zoonotic implications. When dietary manipulation has been used as an adjuvant to drug therapy, it may have beneficial results controlling weight loss, resolving diarrhea, and preventing cyst shedding. The addition of fiber, probiotics, and protectants (intestinal wall protectants or liver protectants) may be also used as co-adjuvants in the treatment of giardiasis (Scorza & Lappin, 2012).

Table 1. Drug therapy used for the treatment of giardiasis in dogs and cats; modified from Tangtrongsup & Scorza, 2010

Active principle	Species	Posology
Metronidazole	Cat and Dog	15 to 25 mg/kg, PO, q12 to 24h, for 5 - 7 days
Tinidazole	Dog	44 mg/kg, PO, q24h, for 6 days
Ipromidazole	Dog	126 mg/L of drinking water, PO, ad-libitum, for 7 days
Fenbendazole	Cat and Dog	50 mg/kg, PO, q24h, for 3 days
Albendazole	Cat and Dog	25 mg/kg, PO, q12h, for 2 days
Pyrantel, praziquantel, febantel	Dog	Label dose, PO, for 3 - 5 days
	Cat	56 mg/kg (based on the febantel component), PO, q24h, for 5 days
Quinacrine	Dog	9 mg/kg, PO, q24h, for 6 days
	Cat	11 m/kg, PO q24h, for 12 days
Furazolidone	Cat	4 mg/kg, PO, q12h, for 7 - 10 days

The nitroimidazoles family, which includes metronidazole, has anti-protozoan properties in humans and animals. Its mechanism of action is damaging the structure of the DNA of the parasite (Miller, Howes, Kasubick, & English, 1970). Metronidazole is well absorbed after oral administration and inhibitory concentrations can be found in many tissues and secretions. Nitroimidazoles are primarily metabolized by the liver and excreted in the urine (Lau, Lam, Piscitelli, & L. Wilkes, 1992). Metronidazole should be administered if concurrent infection with *Clostridium perfringens* is suspected because of the known antibiotic activity against this bacterium (Tangtrongsup & Scorza, 2010; Scorza & Lappin, 2004).

Several studies demonstrate the efficacy of benzimdazoles against *Giardia* (Barr, Bowman, Heller, & Erb, 1993; S. Barr, 1994). The mechanism of action of benzimidazoles is based on the disruption of the architectures of the cytoskeleton microtubules (Navarrete-Vázquez, et al., 2001; Morgan, J.A., & R.C.A., 1993). This drugs has generally broad spectrum of activity and low toxicity (Gokbulut, Bilgili, Hanedan, & McKellar, 2007) Fembendazole, as a known anthelmintic, is recommended for treatment when co-infection with nematodes is suspected (Rossignol, 2010). The combination of pyrantel/praziquantel/febantel can be used as well when co-infection with nematodes is present. Febantel has been demonstrated to be effective for the treatment of dogs and cats with *Giardia*. However, there exist some discrepancies among efficacy studies, probably due to the different formulation used on those studies (Rossignol, 2010; Olson & Heine, 2009).

1.1.9 Prevention

Taking into account the primary mode of transmission of *Giardia* and its associated risk factors, the prophylactic measures to prevent or, at least, decrease the ingestion of infective cysts can be instituted. These preventive measures include:

1. Cyst free environments: maintaining the areas clean from feces will decrease the chance of cyst ingestion. The use of steam cleaners or chemical disinfectants is highly recommended. A 1:30 dilution of 5% sodium hypochlorite or quaternary ammonium used at the manufacturer concentration effectively inactivates *Giardia* cysts. In addition, because the cysts are susceptible to drying, allowing the area to dry after the cleaning is recommended. (Scorza & Lappin, 2012).
2. Cleaning cysts from coats: Grooming is a factor that increases the probability of infection or re-infection. Thus, animals at risk or in treatment may be bathed with regular pet shampoo. In addition, the use of non-irritant disinfectant may be used to clean the perinea area (Scorza & Lappin, 2012).
3. Keep the *Giardia* outside: In the case of large animal populations such as kernels or shelters, it is recommended that new dogs or cats to be bathed, as presented above, regardless if they are *Giardia* negative (Scorza & Lappin, 2012). The fomite transmission is a known way of spreading these infections, thus the use of basic biosecurity measures is recommended (Scorza & Lappin, 2012).

In conclusion, the prevention of *Giardia,* as it is for most of the infectious diseases, is a battleground with different fronts. Thus, the strategic integrated approach is probably the best way to prevent infection or re-infection with *Giardia.*

1.1.10 Public health significance

Current advances in molecular techniques have improved the understanding of the taxonomy and further the assemblage arrangement of *Giardia* isolates among species. This opens the discussion regarding the zoonotic potential of some of those assemblages. Particularly, the assemblage AI have been identified in humans, dogs, and cats (Ballweber, Xiao, Bowman, Kahn, & Cama,

2010; Thomson, Palmer, & O'Handley, 2008; Scorza & Lappin, Giardiasis, 2012).However, to conclude that the zoonotic potential of *Giardia* is a tangible risk, both biological and epidemiological information should be congruent (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010). The molecular techniques of identification have to be analyzed with caution, because the identification of a particular assemblage depends on the chosen genetic marker, thus the multi-locus analysis is more suitable for establishing any actual connection (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010). Also in the review by Ballweber et al. (2010) it is stated that

> *"A robust molecular tool for consistent taxonomic classification and sufficient data on the population genetic structure of G. duodenalis are currently lacking, which are needed to understand more completely the transmission dynamics and zoonotic potential of this parasite."*

This may imply that, with the actual available tools, there is not enough evidence to conclude that human outbreaks of giardiasis comes from animal source or *vise versa*.

Even though, there are some reports indicating that the same type of *Giardia* was found in samples from dogs, cats, and humans interacting closely, there are still uncertainties in the epidemiologic triangle connecting giardiasis from pets to giardiasis in humans, and the pathway of causation is unclear (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010).

1.2 CRYPTOSPORIDIOSIS IN CATS AND DOGS

1.2.1 Etiology

Ernest Edward Tyzzer was the first to name and describe *Cryptosporidium* in 1907 using characteristics such as the host species, location, and morphologic particularities (Fayer, 2010). Since Dr. Tyzzer discover *Cryptosporidium*, the host specificity, location in the host, and morphology characteristic have been the basis for taxonomy classification for species of the phylum Aplicomplexa (Fayer, 2010). From the decade of the 70s until the 90s, it was believed that only one species (*Cryptosporidium muris*) parasitized the gastric mucosa of mammals, while

Cryptosporidium parvum parasitized the intestine of mammals (Fayer, 2010). With the development of novel molecular techniques, it was finally understood that there were two different cycles of transmission related to the genotype: the human (human-to-human) and bovine (animals-to-humans) genotypes (Fayer, 2010). The naming of a new species occurs now if the biological and genetic information is sufficient to identify an isolate as unique (Fayer, 2010).

Below is the taxonomic classification of the genus *Cryptosporidium:*

Kingdom	Protozoa
Phylum	Apicomplexa
Class	Conoidasida
Order	Eucoccidiorida
Suborder	Eimeriorina
Family	Cryptosporidiidae
Genus	*Cryptosporidium* Tyzzer, 1907 (Integrated Taxonomic Information System, 2013).

In 1979, Iseki described *Cryptosporidium felis*, the species that affects mainly cats. In addition, it was reported to be infective in both bovines and humans (Fayer, 2010). *Cryptosporidium muris* was identified in naturally infected cats (Pavlasek & Ryan, 2007). In the same manner, *C. canis* was identified to be the dog genotype and was established as an independent species based on transmission and molecular experiments; this genotype can infect young bovine as well (Fayer, 2010).

1.2.2 Morphology

The typical zoites (merozoites or sporozoites) of *Cryptosporidium* are similar to other apicomplexans; they present crescent shaped cell body, apical rhoptry and micromeres, and

dense granules distributed throughout the cytoplasm **¡Error! No se encuentra el origen de la referencia.** (O'Hara & Chen, 2011). The parasite surface (pellicle) is a multilayer membrane; the outer and inner membranes are each composed from two membranes and sub-pellicular microtubules (O'Hara & Chen, 2011).

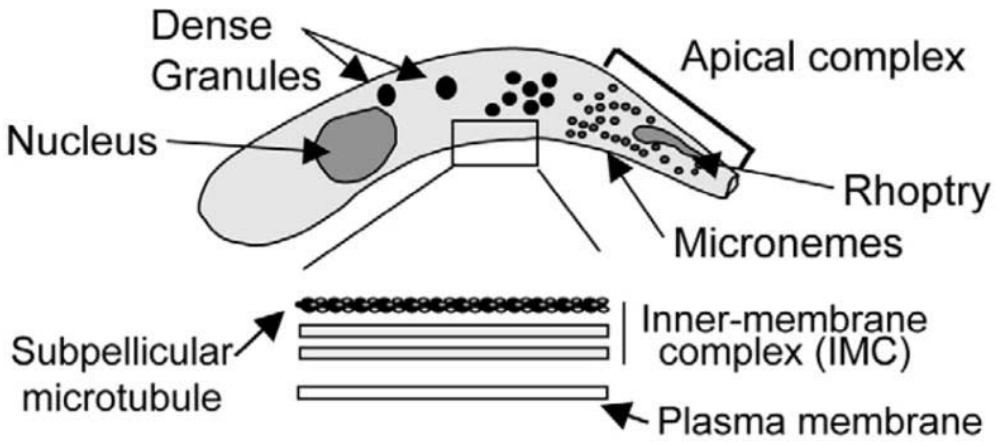


Figure 4. Scheme of the morphologic characteristics of a *Cryptosporidium* zoite. (Elsevier license 3416010271077)

The endogenous stages of the parasites are closely associated with the luminal surface of the epithelial cells; they protrude from the cell surface. These bodies have spherical or elliptical shapes with sizes ranging from 2 to 6µm. Their location has been determined to be intracellular but extra-cytoplasmic within the parasitophorus vacuoles membranes (O'Donoghue, 1995). The pellicle folds repeatedly forming a structure that adheres to the microvilli (O'Hara & Chen, 2011).

The oocyst is the exogenous, infective, and environmental-resistant form of the parasite. Mature oocysts contain 4 sporozoites enclosed within a oocyst. This configuration provides some of the characteristics for its visual classification. The oocysts vary in size and shape depending on the species, ranging from 4.5 to 8 µm in length by 4 to 6.5 in width (O'Donoghue, 1995).

1.2.3 Life cycle

After ingestion of the infective oocyst, excystation of the four sporozoites is triggered mainly by the change in temperature and pH. The sporozoites migrate along the surface of the epithelium until they find a place to attach. This process is driven by a complex biochemical mechanisms that include interaction of *Cryptosporidium* sporozoites with the host cell's cytoskeleton. This process has been called gliding motility (Wetzel, Schmidt, Kuhlenschmidt, Dubey, & Sibley, 2005; O'Hara & Chen, 2011). The formation of the parasitophorus vacuole occurs after being encapsulated by a parasite modified host membrane. This process is known as internalization (O'Hara & Chen, 2011). During internalization, the feeder organelle is formed between the parasite and host cytoplasm. This organelle confers selective transport properties between host and parasite for nutrients uptake (O'Hara & Chen, 2011).

Type I, followed by TypeII meronts develop next. These are derived from the asexual reproduction of the trophozoite in the process known as endopolygeny. The formation of the daughter cells occurs while still in the mother cell (O'Hara & Chen, 2011). The type I meront produce merozoites that are morphologically and biologically similar to the sporozoites. These merozoites invade the surrounding enterocytes and can produce meronts type I and II (O'Hara & Chen, 2011; Scorza & Lappin, 2012).

Merozoites, derived from Type II meronts, differentiate into gametocytes to complete the sexual stage of development. These gametocytes can be either male or female reproductive stages, known as microgametocyte and macrogametocyte respectively (O'Hara & Chen, 2011). The fertilizationof the macrogametocyte by the microgametocyte results in the only diploid stage of development (the zygote), which undergoes sporogony process (meiosis-like process) resulting in the production of a sporulated oocyst containing four sporozoites. This oocyst can be thin or

thick-walled, the thick-walled oocysts are shed in the feces, and the thin-walled oocyst excysts within the intestinal lumen starting a process of autoinfection and escalating the infection level (O'Hara & Chen, 2011).

1.2.4 Pathogenesis

After excystation process, the free sporozoites adhere to the mucous membrane of the small intestine by a carbohydrate-lectin mediated mechanism (O'Hara & Chen, 2011). Multiple proteins, localized in the apical surface of the zoite, have been identified to be importantly involved in the attachment process; gp40, gp15, gp900, and Circumsporozite-like glycoprotein (CSL) are some (O'Hara & Chen, 2011). Furthermore, a Gal/GalNAc-specific lectin (p30) was identified having lectin activity. Another sporozoite protein (cp47) localized in the apical region of the parasite, was found to be highly correlated with the efficiency of in vitro infectivity. It has been demonstrated that this protein interacts with a 57kDa (p57) protein of the host cell which is abundant in the ileum. This explains, in part, its affinity for this tissue (O'Hara & Chen, 2011).

The motility possess of aplicomplexans undergoes a unique method that is defined by the absence of any obvious modification of the shape of the moving cell (O'Hara & Chen, 2011; Smith, Nichols, & Grimason, 2005). The structural stability and polarity is maintained by the microtubules, while the locomotion and invasion mechanism is provided by the actomyosin system. The investigation of the gliding mechanisms in *Toxoplasma gondii* and *Plasmodium* have shown that trophozoites left a trail of proteins that are released (shed) trough the posterior pole of the cell (O'Hara & Chen, 2011; Smith, Nichols, & Grimason, 2005). The process of gliding motility, then, comprises three main steps: i) the secretion of adhesive molecules from the apical pole of the parasites that adhere to the host cell receptors; ii) the posterior translocation of the adhesive

molecules; and iii) the proteolytic cleavage and release of the parasite molecules in motility trails (O'Hara & Chen, 2011).

After the zoite has found its niche in the luminal surface of the host, the process of invasion is initiated by the fusion of both parasite and host membranes. The rhoptry is in close relation with the site of attachment and other organelles associated with the process (micronemes and dense granules) migrate to the parasite-host interface. The cytoplasm of the zoite vacuolize and a tunnel-like structure is formed in this location (O'Hara & Chen, 2011; Smith, Nichols, & Grimason, 2005). The process of internalization-invasion starts with the clustering of vacuoles that ultimately encloses the parasite. A unique condition is derived from this process; the zoites remains extra-cytoplasmic yet intra-membranous (intracellular) (O'Hara & Chen, 2011). In addition, a structural support is formed at the base of the parasite-host interface by a network of recruited host actin (O'Hara & Chen, 2011). After internalization, the parasite also recruits the host cell channels and transporters to the parasite-host interface, which further serve to nourish and support the sporozoite (Smith, Nichols, & Grimason, 2005). .

It was demonstrated the altered expression of over 200 genes in infected cultured human cells; the main altered genes include those associated with apoptosis, cyto-skeletal dynamics, and pro-inflammatory signaling cascades (O'Hara & Chen, 2011). One of the most important mechanisms for the proliferation of the infection is the inhibition of apoptosis, because the parasite requires viable host cells for the completion of its life cycle (O'Hara & Chen, 2011). Perhaps, the epithelial cell apoptosis mechanism is protective, limiting the parasites number (O'Hara & Chen, 2011).

The loss of epithelial brush in cryptosporidiasisis most likely caused by the immune host response rather than by any direct effect of the parasite (Scorza & Lappin, 2012).

1.2.5 Epidemiology

Cryptosporidium is distributed throughout the world. Its transmission is related to crowded and unsanitary conditions; immunocompromised individuals are specially affected by this kind of parasites (Fayer, 2010; O'Donoghue, 1995).

The prevalence of *Cryptosporidium* in dogs and cats is variable throughout the different reports (**¡Error! No se encuentra el origen de la referencia.**). The variation of these findings may be due, at least in part, to the uses of different tests that have different detection thresholds or in other words different sensitivity and specificity values, In such reports the number of true positive or true negative is unknown, which makes, the necessity for a reference test, even more evident.

Table 2. Prevalence of *Cryptosporidium* in dogs

Prevalence	Country	Method	Environment	Reference
2%	USA CA	Auramine-rhodamine fluorescent staining procedure.	Shelter	(el-Ahraf, Tacal, Sobih, Amin, Lawrence, & Wilcke, 1991)
7.4%	Spain	Ziehl-Neelsen	Veterinary clinic and Animal shelter	(Causapé, Quílez, Sánchez-Acedo, & Cacho, 1996)
9.3%	Japan	PCR	Stray dogs	(Niichiro Abe, 2002)
3.8%	USA CO	IFA (Merifluor)	Veterinary clinic	(Hackett & Lappin, 2003)
3.3%	Italy	PCR	Private owners and Kernels	(Giangaspero, Iorio, Paoletti, Traversa, & Capelli, 2006)
1.4%	Czech Republic	Ziehl-Neelsen	Urban and Rural	(Dubná, et al., 2007)
2.2%	Brazil	Methylene blue gram	Kernels	(Mundim, Rosa, Hortêncio, Faria, Rodrigues, & Cury, 2007)
3.1%	Brazil	Ziehl-Neelsen	Private owners and Kernels of Sao Paulo	(Katagiri & Oliveira-Sequeira, 2008)
5%	Iran	Ziehl-Neelsen	Private owners Rural	(Beiromvand, et al., 2013)
3.8%	China	Concentration and light microscopy	Average	(Jian, et al., 2014)
7%			Kernels	

The main way of transmission is the fecal-oral route; by ingestion of the infective oocysts contaminating water or food sources, grooming, or the ingestion of infected preys (Scorza & Lappin, 2012). The oocysts are resistant to several environmental conditions, as well as common

disinfectants (Scorza & Lappin, 2012). In human populations, the contamination of public water supplies can lead to large outbreaks of cryptosporidiosis (Scorza & Lappin, 2012)

The risk factors associated with cryptosporidiosis in humans includes contact with contaminated water (recreational or drinking), exposure to infected animals (mainly bovines), travel to disease endemic areas, and ingestion of contaminated food (Yoder & Beach, 2010). Groups implicated with higher risks of infection include children and staff in day care centers, farmers and animal handlers, health care workers, and travelers to endemic zones (Ramirez, Ward, & Sreevatsan, 2004). Other commonly mentioned factor that increases the risk for cryptosporidiosis is the pretense of any type of immunodeficiency including but not limited to HIV infection and AIDS, drugs, organ transplantation, cancer chemotherapy, etc (Ramirez, Ward, & Sreevatsan, 2004). In pets, cats specifically, some of the reported associated factors are age (<1 year), presence of other enteric parasites (*Giardia*), feeding with not commercial diet, and diarrhea (Luisa Rambozzi, 2007; Ballweber, Panuska, Huston, Vasilopulos, Pharr, & Mackin, 2009). In a study in dogs in the province of Rio de Janeiro, Brazil, sporadic diarrhea and vomiting, living with cats, and the owner socioeconomic status were found to be significantly associated with canine cryptosporidiosis (Ederli, Ederli, Oliveira, Quirino, & Carvalho, 2008).

1.2.6 Clinical findings

Many infections caused by *Cryptosporidium* in cats and dogs are subclinical or cause only mild clinical sign (Scorza & Tangtrongsup, 2010). The most common clinical signs associated with cryptosporidiosis are small bowel diarrhea, anorexia, and weight loss (Scorza & Lappin, 2012; Scorza & Tangtrongsup, 2010). In some animals, particularly animals affected by any type of immunodeficiency (viral, iatrogenic, stress, malnutrition, etc.) or co-infection with other enteric parasites, the infection may cause chronic diarrhea and malabsorption syndrome (Scorza &

Tangtrongsup, 2010). For some cases, it is difficult to establish if *Cryptosporidium* is the primary cause of the clinical signs, because of the presence of other etiologies: other parasites, viral infections, bacterial infections or inflammatory processes (Scorza & Lappin, 2012).

1.2.7 Diagnosis

As well as for diagnostic of *Giardia*, there are a number of available laboratory techniques for the detection of *Cryptosporidium*, which are summarized in the following section.

1.2.7.1 Conventional Microscopy

The direct microscopic examination of wet mounts is not used regularly. Even with the addition of concentration, the recognition of the oocysts in direct mounts is difficult due to the small numbers of oocysts in the feces of dogs, cats, and humans and can lead to false negative results. For this reason, Use of staining procedures can be used to increase the sensitivity of the microscopic tests; the most frequently used staining techniques are modified Ziehl-Neelsen (MZN) acid fast, safranin-methylene blue stain, Kinyoun acid fast, and DMSO-carbol fushin. With the MZN staining, the oocysts are stained with carbol-fuchsin and the dye is retained in the decolorizing step with acid alcohol. One major disadvantage of this technique is its low sensitivity (70%) (Marks, Hanson, & Melli, 2004). However, this is a test that can be performed in small practices with a light microscope, and can serve as initial screening test (Scorza & Tangtrongsup, 2010).

1.2.7.2 Immunochemical antigen detection

Some commercial DFA tests are available for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts. This technique can be more sensitive and specific than other microscopic techniques; its detection threshold is as low as 10^4 oocysts/gram of concentrated feline feces (Scorza, Brewer, & Lappin, 2003; Scorza & Lappin, Giardiasis, 2012; Weber, Bryan,

Bishop, Wahlquist, Sullivan, & Juranek, 1991). As with *Giardia*, one of its advantages is that the results are based not only on the specific antibody link but also in the recognition of the morphology by the examiner. One of the major disadvantages is that this technique requires the use of a microscope with a fluorescent lamp for the examination of the slides, which is not often a regular equipment in private practices.

A number of ELISA tests for the detection of *Cryptosporidium* fecal antigens are available for use with human feces. One; one of its major advantages these techniques is that does not require the use of complex equipments or specific training. In kits, the readings of results can be performed by the comparison of the colorimetric change against a scale. On the other hand, most of the available commercial ELISA tests used in veterinary medicine have been developed for the diagnosis of cryptosporidiosis in humans, with *C. parvum* as its principal target. Antigenic differences amongst *C. parvum*, *C canis*, and *C. felis* exist which can explains why the results of these assays when used with dogs or cat feces are inconsistent (Scorza & Tangtrongsup, 2010; Marks, Hanson, & Melli, 2004).

1.2.7.3 Molecular techniques

The use of molecular techniques has help to elucidating the complex research questions about the biology, taxonomy, pathogenesis, and epidemiology of *Cryptosporidium* (O'Hara & Chen, 2011; Thomson R. , 2004; Fayer, 2010). Moreover, the use of molecular techniques for detection of oocysts has been increasing (Scorza, Brewer, & Lappin, 2003). The amplification of *Cryptosporidium* DNA in feces can be a useful tool. This has shown to be more accurate than ELISA tests and Ziehl-Neelsen (Uppal, Singh, Chadha, & Jha, 2014; Omoruyi, Nwodo, Udem, & Okonkwo, 2014; Scorza, Brewer, & Lappin, 2003). In addition, when the sequencing is added to the analysis of samples, the association of particular species with the infection can be determined

. (Thomson R. , 2004). However, since these kinds of techniques are more expensive and lack from extensive or well-controlled studies that allow us to estimate its performance. The use of this technique has been limited to identification of cases with chronic-unexplained diarrhea that are negative to other tests, or when genotyping is the goal (Scorza & Tangtrongsup, 2010).

1.2.7.4 Other diagnostic tools

Other possible available tools for the diagnosis of *Cryptosporidium* are detection of serum antibodies (ELISA or FA), inoculation of mice, and intestinal biopsy; nonetheless, those techniques are not being used routinely in the diagnostic laboratory (Scorza & Tangtrongsup, 2010). Immuno-PCR is a technique has been used for detection of low concentration of oocyts in water sources. This technique is based on the primary attachment by antigen-antibody complexes to a gold matrix that afterwards is used to perform PCR (Deng, et al., 2014).

1.2.8 Treatment

Over 100 compounds have been evaluated for the treatment of cryptosporidiosis. However, none of them has shown clear remission of signs or elimination of infection (Scorza & Lappin, 2012; Rossignol, 2010). Thus, the primary goal of the treatment should be to stop diarrhea. Palliative support should be given, according to practitioner discretion. The use of high digestible diet, hydration solutions, mucosal protectors, and antibiotic for secondary bacterial infection may be necessary as part of the treatment of cryptosporidiosis (Scorza & Tangtrongsup, 2010). Chemotherapy in cats and dogs lacks of extensive studies showing the efficacy therapy to control the clinical signs of *Cryptosporidium* infection. . (Scorza & Tangtrongsup, 2010; Thomson, Palmer, & O'Handley, 2008; Armson, Reynoldson, & Thompson, 2003). In companion animals, positive results have been reported for treating infections with *Cryptosporidium* using paromomycin, tylosin, or azithromycin (Lappin, 2004). Table 3, modified from Scorza &

Tangtrongsup (2010), shows the treatment porotocols used on cats and dogs with cryptosporidiosis.

Table 3. Table 1Drug therapy used for the treatment of cryptosporidiosis in Dogs and Cats; modified from Scorza & Tangtrongsup (2010).

Active principle	Posology what is this?
Azithromycin	10 mg/kg, PO, q24 hours, until remission of clinical signs.
Nitazoxanide	25 mg/kg, PO, q12 hours, for at least 7 days.
Paromomycin	125 - 165 mg/kg, PO, q12 - 24 hours, for at least 5 days.
Tylosin	10 - 15 mg/kg, PO, q8 - 12 hours, for 21 days.

Paromomycin is an antibiotic, part of the amino-glycoside group; its mechanism of action is based on the disruption of the protein synthesis pathway targeting the ribosome (Gargala, 2008). Its absorption is limited at the intestinal level, but can be absorbed in small amounts at the apical membrane of the epithelial cell (Gargala, 2008; Scorza & Lappin, 2012). Paromomycin has been evaluated in cats, showing decreased oocyst shedding to below detection limits (Scorza & Tangtrongsup, 2010; Lappin, 2004). When there is uncertainty of the integrity of the mucosal membrane, however, its use should be avoided, because of increased absorption rates, which result in renal and ototoxicity (Scorza & Tangtrongsup, 2010).

Azithromycin is an azalide antibiotic, which interferes with the microbial protein synthesis, and is considered the most active among the macrolides (Gargala, 2008). Azitrhtomicin has been evaluated in animals. It has been reported that the administration to infected calves, improves the clinical signs and reduces the oocyst shedding (Elitok, Elitok, & Pulat, 2008).

Nitazoxanide (NTZ) is a 5-nitrothiazolyl salicylamide derivative with well-known activity against protozoa and helminthes (Gargala, 2008). NTZ has been administered to cats and dogs resulting in remission of clinical signs. However, NTZ also causes intestinal irritation, and it is not effective when the patient is not immuno-competent (Scorza & Tangtrongsup, 2010)

Tylosin has been administrated to cats and dogs empirically resulting in improvement of clinical signs. However, these observations were uncontrolled and it is possible that the results of tylosin administration were related to the control of bacterial co-infection or anti-inflammatory effects. In addition, tylosin can be a gastrointestinal irritant and it is not well tolerated by cats because of its taste (Scorza & Tangtrongsup, 2010; Westermarck, et al., 2005).

1.2.9 Prevention

Cryptosporidium oocysts are resistant to extreme temperatures and most frequently used disinfectants. Concentrated ammonia solution (50%) has been effective for inactivation of oocysts. Steam (>55°C), freezing thawing, and drying are effective preventive measures for the inactivation of oocyts (Scorza & Tangtrongsup, 2010).

Cryptosporidium oocysts and *Giardia* cysts have similar characteristics of resistance to the environmental conditions. Both agents share many epidemiologic features and thus the measures of control may be work for preventing their infection. Furthermore, maintaining the areas clean from feces plus the use of chemical disinfectants, and low humidity floors will decrease the chance of oocyst ingestion. Quarantine or isolation may be recommended for infected individuals. Suspected animals may be bathed with regular pet shampoo to decrease the risk of infection by grooming. Screening test and regular baths are recommended for new members of a population.

1.2.10 Public health significance

In the past, it was believed that each *Cryptosporidium* species or genotype infects a particular host species. *Cryptosporidium parvum* was considered to infect humans, but later, with the inclusion of genotyping techniques, *C. parvum* was separated into two genotypes: *C. parvum*—the bovine genotype, and, *C. hominis* infecting only humans (Thomson, Palmer, & O'Handley,

2008). Additionally, the species affecting cats and dogs (*C. felis* and *C. canis*) have been identified in human samples. However, the zoonotic roll *C. felis* and *C. canis* seems to be limited, because the infection rates of those species in humans are low (0.26% and 0.02% respectively), and many studies have failed to show strong association between human cryptosporidiosis and pet contact (Scorza & Tangtrongsup, 2010; Lucio-Forster, Griffiths, Cama, Xiao, & Bowman, 2010). In the case of HIV-infected people, it should be recommended to avoid any contact with infected pets, and the sanitization practices should be emphasized in order to decrease the risk of transmission (Scorza & Lappin, 2012; Scorza & Tangtrongsup, 2010; Lucio-Forster, Griffiths, Cama, Xiao, & Bowman, 2010).

1.3 DIAGNOSTIC TEST ASSESSMENT

1.3.1 Notation and definitions

In this review, the terms *probability* and *proportion* are used synonymously and will be defined by relative frequency. Let *A* denotes the event that a randomly selected subject from a population has a defined characteristic. *N* denotes the total number of people in one population, thus N_A the number of subject that has characteristic *A*. Then *P*(*A*) denotes the proportion of all subjects that have the *A* characteristic or, likewise, *P*(*A*) is the probability that a randomly selected subject has the characteristic *A*; $P(A) = N_A/N$. Thus *P*(*A*) should be a real number contained between 0 and 1 ($0 \leq P(A) \leq 1$). Let $P(\bar{A})$ denotes the proportion of subjects that do not have the characteristic *A*, then $P(\bar{A}) = N_{\bar{A}}/N$ and $P(\bar{A}) = 1 - P(A)$, so $P(\bar{A})$ is denominated the complementary proportion to one of *P*(*A*). In the same manner, if N_B denotes the number of subjects that have characteristic *B*, $P(B) = N_B/N$, and its complementary $P(\bar{B}) = N_{\bar{B}}/N = 1 - P(B)$.

Additionally, if N_{AB} is the number of subjects that have both characteristics, we can describe the proportions of subjects having these two characteristics at the same time as $P(A\ and\ B) =$

N_{AB}/N. The so-called *conditional probability* is defined by $P(A|B)$, which is the probability that a randomly selected subject has a characteristic *A* given that it has characteristic *B*, or is *conditional* on having characteristic *B*. As stated above, $P(A \text{ and } B)$ represent the proportion of all subjects that possess both characteristic *A* and characteristic *B*, then

$$P(A|B) = \frac{N_{AB}/N}{N_B/N} = \frac{N_{AB}}{N_B} = \frac{P(A \text{ and } B)}{P(B)},$$

similarly

$$P(B|A) = \frac{N_{AB}/N}{N_A/N} = \frac{N_{AB}}{N_A} = \frac{P(A \text{ and } B)}{P(A)}.$$

The *association* of two characteristics means that the probability of having one characteristic is affected by the probability of having other characteristics. In contrast, the *independence* or lack of association of two characteristics means that given that the subject have one characteristic does the probability of having the other characteristic is not affected. Then,

$$\frac{P(A \text{ and } B)}{P(B)} = P(A) \Rightarrow P(A \text{ and } B) = P(A) \times P(B).$$

This equation is often taken as the definition of independence.

When two proportions are matter of studies, the aim is often to establish or discard any type of association. The inclusion of the conditional proportion to the equation allow for that as

$$P(A \text{ and } B) = P(A|B) \times P(B).$$

With the rule of *total probability*, it is possible to know the probability of having one characteristics including conditional and complimentary probabilities as

$$P(B) = P(A \text{ and } B) + P(B \text{ and } \bar{A}),$$

then

$$P(B) = P(B|A) \times P(A) + P(B|\bar{A}) \times P(\bar{A}).$$

The *Bayes' Theorem*, from the frequentist point of view, connects the conditional probabilities of *A* given *B* and vice versa by the probabilities of each event, this is

$$P(B|A) = \frac{P(A|B)P(B)}{P(A)}.$$

Bayes' Theorem is a theorem of probability theory, it was originally stated by the Reverend Thomas Bayes. We can explain it as the way of how the probability of a true event can be affected by the inclusion of the probability of other event as a piece of evidence (Feiss, Levin, & Paik, 2003).

1.3.2 Applied probability for diagnostic tests

For this part of the review, let *T* denotes the positive result of a diagnostic test, then $\overline{T}$ denotes the complimentary negative result. In the same way, let *D* denotes the presence of disease in an individual, and $\overline{D}$ its complimentary absence of disease indicator.

The *sensitivity* of a test, denoted *Se*, is the probability that a true positive or diseased sample tests positive, thus, following the probability notation above,

$$Se = P(T|D).$$

The *specificity* of a test, denoted *Sp*, is the probability that a true negative or non-diseased sample tests negative, thus

$$Sp = P(\overline{T}|\overline{D}).$$

The disease *prevalence* (true prevalence) in the source population, denoted *Pr*, is the proportion of subjects from the source population that have the disease, thus

$$Pr = P(D).$$

The term *apparent prevalence* (*APr*), is given to the proportion of subjects that have positive test result,

$$APr = P(T),$$

The predictive value is calculated using the Bayes' Theorem, for those subjects with positive test results this probability is called the Positive Predictive Value (PPV), defined by:

$$PPV = P(D|T) = \frac{P(T|D)P(D)}{P(T)} = \frac{Se \times Pr}{Se \times Pr + (1 - Sp)(1 - Pr)}.$$

Knowing the *Se* and *Sp* and *Pr*, the rule of total probability is used to obtain the proportions of positive results or apparent prevalence, this is

$$P(T) = P(T|D)P(D) + P(T|\bar{D})P(\bar{D}) = Se \times Pr + (1 - Sp)(1 - Pr).$$

In the same way, the Negative Predictive Value (NPV) is defined by

$$NPV = P(\bar{D}|\bar{T}) = \frac{P(\bar{T}|\bar{D})P(\bar{D})}{P(\bar{T})} = \frac{Sp \times (1 - Pr)}{Sp \times (1 - Pr) + (1 - Se)Pr}.$$

Confidence intervals (CI) can be calculated using the formula for estimating the Standard Error (SE) and the CI for a single proportion

$$SE(p) = \sqrt{\frac{p(1-p)}{N}},$$

then

$$\theta \mp Z_{1-\propto/2} \times SE(p),$$

where *p* is the proportion or probability of interest, *N* is the number of subjects of interest, θ is the upper or lower CI, and $Z_{1-\propto/2}$ is the $1 - \propto/2$ percentile of the normal distribution.

The sample size for the estimation of Pr, Se and Sp would depend on the desired confidence level for the estimates and the allowed error in the estimates, then for estimation of the number of samples (*n*) the formula is

$$n \geq p(1-p) \times \frac{Z^2_{1-\propto/2}}{e^2},$$

where *p* would be replaced for the decent guess of the parameters (*Pr, Se* ,or *Sp*) and *n* represents

the total number of samples, number of positive samples, or number of negative samples for estimation of *Pr, Se,*or *Sp* respectively.

1.3.3 Diagnostic performance

The performance of a diagnostic test is described by two independent measurements: precision and accuracy. *Precision* refers to the ability of the test to produce consistent results within tolerable analytical error limits. In the other hand, the *accuracy* (syn. *validity*) refers to the ability of the test to produce correct test results (Greiner & Gardner, 2000).

The Sensitivity (Se) and Specificity (Sp) are the primary validity indicators of a test; these indicators are the base for further calculations, interpretation, and decision-making. Ideally, those values are deriving from testing a group of samples from reference subjects, with known particular relevant disease status (i.e. known diseased and known non-diseased subjects) (Jacobson & Wright, 2013). In the same way, this assessment, of the true state of disease can be achieved by testing the reference population with a ***reference test*** or ***gold standard***, which results represent the true disease state of that population (TDR Diagnostics Evaluation Expert Pannel, 2010).

It is commonly observed that Se and Sp vary among published studies. This variation could be attributable to differences among the reference populations and sampling strategies (Greiner & Gardner, Epidemiologic issues in the validation of a veterinary diagnostic tests, 2000). Other sources of variation can be related to the technical variation of the test (cut-off points, reference population, reagents, etc.) (Greiner & Gardner, 2000). The logistic and financial issues are usual limitations for the optimal estimation of validity indicators, since many samples are required to achieve high confidence levels (Jacobson & Wright, 2013).

Sensitivity and Specificity can be calculated when the outcome is continuous or measured in ordinal scale, which requires the definition of a **cut-off** point (Greiner, Pfeiffer, & Smith, 2000).

Cut-off points can be determined in different ways: it can be arbitrary defined as two or three standard deviations greater than the mean of the results for the unaffected subjects. Alternatively, it can be defined as the value that minimizes the cost or number of misdiagnosed subjects. In addition, there are statistical approaches to define the optimal cut-off point; the likelihood ratio and the ROC curves are some of them (Greiner, Pfeiffer, & Smith, 2000). It is accepted that there are certain arbitrariness in assessing the estimates of validity when the binary outcome is extracted from a continuous result, then the probability of misclassifying a true positive as negative tends to be higher for those samples with values that are nearer to the cut-off point (Szklo & Nieto, 2007). Thus, the Se and Sp of a test based on a cut-off in a continuous distribution, depend on the distribution of the severity of the condition (Szklo & Nieto, 2007).

The values of Se and Sp should be considered in the context of a particular diagnostic objective. The cost of false positive and/or false negative results needs to be accounted in the decision making process. Below is a brief description of two main scenarios where a diagnostic test can be used to asses uncertainty and help the decision maker(s), taking in account the possible outcome of the decision.

The first scenario can be described as the typical clinical case that is attended by a practitioner in any health center. In this particular case, the practitioner tries to asses as much information as possible in order to decrease the probability of misclassifying the diagnosis. A misdiagnosed false negative implies that a true diseased patient would not receive the proper treatment and its problem would not be fixed. In the other hand, a false positive result implies that a patient would receive a treatment being non-diseased and may have to deal with the potential side effects of the treatment. In addition, the initial problem or consultation motif would not be accurately

addressed or solved. Most of the times practitioners prefer a more specific test since the cost of false negative results may be higher (Lilford, Pauker, Braunholtz, & Chard, 1998).

The second main scenario is when the diagnostic test is intended to assess a particular trait in a population. The diagnostic tests receive a different denomination, most scientific sources refer to this as a ***screening test***. Health certification schemes, risk-factor studies, risk of disease introduction, and disease-control programs are some of the scenarios where a screening test would be used. In these situations, the intervention is usually determined by the status of the entire population rather than by the status of each individual within the population. The presence of a false negative result in a particular population may imply that a true infected individual would not be detected and no control measure would be taken. Therefore, for a transmissible disease, the disease can spread unnoticed. In the other hand, if a false positive result is delivered, a non-diseased animal may be treated, quarantined, sold, evacuated, or sacrificed as part of the control measures. Additionally, this population may lose disease-free certification, incurring unnecessary extra management costs. In contrast, with the clinical case scenario, it is preferable to use a test with high sensitivity rather that a more specific one, since the cost of managing a false negative would be relatively higher (Christensen & Gardner, 2000).

The true prevalence (*Pr*) and the apparent prevalence (*APr*) are important descriptors of the tested subgroup. While *Pr* denotes the actual level of disease that is present, the *APr* is based only on the imperfect positive results of a test, and provides an approximation to the true prevalence (e.g. sero-prevalence).

The predictive ability of the test can be measured by calculating the predictive values of positives and negatives. Once a test has been evaluated in its accuracy, the probability that the individual has or does not have the disease in question should be considered. Furthermore, this

predictive value can be defined by the proportion of diseased (non-diseased) subjects in a population depending on whether the test is positive (negative) (Sox, 1996).

The positive predictive value (PPV) is the probability that a subject has the disease given the test result is positive; in other words, PPV is the proportion of subjects with a positive test that have the disease. In a similar manner, the negative predictive value (NPV) can be interpreted as the probability that a subject does not have the disease given the test result is negative, or the proportion of subjects with a negative test result that do not have the disease.

As was explained above in a mathematical manner, the predictive values are dependent on the *Se* and *Sp* of the test, as well as on the *Pr* of the disease in the population of interest. Since it is accepted that the *Se* and *Sp* are relatively stable for a given reference population (and a given cut-off point), then the prevalence can be the changing variable affecting the predictive values (Smith & Slenning, 2000). In this scenario, if the prevalence is high, the probability that a given positive result represent a true positive (PPV) is higher and the probability that a given negative result represent a true negative is lower, and vise versa (Smith & Slenning, 2000).

The Likelihood ratio (*LR*) provides a summary measure independent of the prevalence. It compares the proportion of animals with and without disease, in relation to their test results (Trustfield, 2005). There are two *LR*s: one for positive results (LR+) and one for negative results (LR-); the LR+ is the ratio of the proportion of diseased individuals with positive results, and the proportion of non-disease individuals with positive results, using the above notation,

$$LR^{+} = \frac{P(T|D)}{P(T|\bar{D})} = \frac{Se}{1 - Sp}.$$

This ratio can be interpreted as how likely is a positive sample to come from a diseased subject than from a non-diseased one. In similar manner, the LR- is the ratio of the proportion of diseased individuals given a negative tests, and the proportion of individuals given a negative tests,

$$LR^{-} = \frac{P(\bar{T}|D)}{P(\bar{T}|\bar{D})} = \frac{1 - Se}{Sp}.$$

This ratio can be interpreted as how likely is a negative sample to come from a diseased subject than from a non-diseased one. The CI for these indicators can be calculated by:

$$\exp\{Ln(LR) \mp Z_{1-\propto/2} \times SE[Ln(LR)]\},$$

where

$$SE[Ln(LR +)] = \sqrt{\frac{1 - Se}{Se \times N_D} + \frac{Sp}{(1 - Sp)N_{\bar{D}}}},$$

and

$$SE[Ln(LR -)] = \sqrt{\frac{Se}{(1 - Se)N_D} + \frac{1 - Sp}{Sp * N_{\bar{D}}}}.$$

Since these *LR*s are functions of the Se and Sp, they are also relatively stable within the reference population (Trustfield, 2005).

There are several others indicators that can be used for measuring the performance of a diagnostic or screening tests. The Cohen's *Kappa* indicator is one of the most common ones. This measures the level of agreement between two sets of test results; beyond what level of agreement would be expected by chance (Cohen, 1960).

1.3.4 Practical applications of diagnostic tests

Diagnostic tests play major roles in veterinary medicine and its applications includes surveillance activities, certification of freedom of disease, prevalence estimation, risk assessment, and other epidemiologic research (Greiner & Gardner, 2000)

The surveillance and monitoring of animal populations, with the aim of detecting or investigating the potentials animal health and productive traits, relies in the diagnostic test. The information received from such results, are critical for the construction of decision-making trees contributing

to the control and prevention of health and productive threats (Greiner & Gardner, Application of diagnostic tests in veterinary epidemiologic studies, 2000).

Establishing the base line of the prevalence of a disease is, many times, a necessary step in the research pathway of ecology of diseases. Not only is the estimation of the frequency of a disease in a population a primary aim of a prevalence study, but, also the estimation of prevalence of protective immunity or exposure to a risk factor (Greiner & Gardner, 2000). From the prevalence or apparent prevalence estimations, control measurements for the population of interest can be inferred. According to this, it is recommended that estimations of prevalence, to be valid as a major information income, should be based on systematic or random representative sampling (Greiner & Gardner, 2000).

For risk-factor assessment, the diagnostic test results may serve as indicator of exposure and as outcome variable. For measuring exposure, a diagnostic test measures the exposure to a particular infectious agent, which is suspect of causing health or economical threats. When the outcome is considered as indicator, the estimates of prevalence, and one or several hypothesized are included in the analysis (Greiner & Gardner, 2000).

The diagnostic test indicators are used as important inputs for the modeling of multiple epidemiologic scenarios. The information used to feed these models may come from different sources: experimental assessment, previous studies, even experts opinions are very valuable. One example of these types of analyses is the assessment of potential quantitative risk in scenario-pathway analyses. These sorts of analyses utilize a series of hypothetic scenarios to model and quantize risk based on previously known information (e.g. quantification of risk from inclusion of infected animals to a susceptible population, fails in the monitoring or surveillance strategies, etc.) (Greiner & Gardner, 2000).

1.4 ASSESSMENT OF DIAGNOSTIC TESTS WHEN THE TRUE DISEASE STATUS IS UNKNOWN

In several cases, the accessibility to a perfect reference tests (gold standard) or to a reference, population is limited or impossible. Then, the true disease state is rarely known in practice and the assessment of the performance of a new diagnostic test becomes a difficult task. Several statistical techniques are available to estimate the tests-performance indicators as well as the populations' prevalence of disease.

In some cases, a non-perfect reference test, with presumed known Se and Sp, is available. The estimates of Se and Sp for the new test and the population prevalence (Pr) are calculated as follow, using the notation in the Table 4:

Table 4. Test results states as positive (T+) or negative (T-). From Enoe, Geordais, & Johnson, 2000.

	Test 2		
Test 1	T+	T-	
T+	*a*	*b*	*g*
T-	*c*	*d*	*h*
	e	*f*	*n*

Thus,

$$\widehat{Se_2} = \frac{gSp_1 - b}{n(Sp_1 - 1) + e}, \widehat{Sp_2} = \frac{hSe_1 - c}{nSe_1 - e}, \hat{P} = \frac{g(Sp_1 - 1) + e}{n(Se_1 + Sp_1 - 1)}.$$

This method can be used under the assumption that knowledge of the outcome of the reference test gives no information about the outcome of the new test conditional on the true disease state (conditional independence) (Enoe, Geordais, & Johnson, 2000).

Hui and Walter (1980) introduced a Maximum Likelihood (ML) model for the estimation of Se and Sp of two tests, based on their cross-classified results, when applied to two populations with different disease prevalence. In addition to the conditional independence, Hui and Walter also

assumed that the Se and Sp values are stable between populations (Enoe, Geordais, & Johnson, 2000). Sampled data from each two (or more) populations are cross-classified in 2 × 2 tables. Each 2 × 2 table provides three degrees of freedom (d.f.) for estimation, then for two populations there are 6 d.f. for estimation (Enoe, Geordais, & Johnson, 2000). It means the number of unknown parameters is six: Se of test 1, Se of test 2, Sp of test 1, Sp of test2, Pr of population 1, and Pr of population 2. Formulas for the ML Estimates (MLEs) were given by Hui and Walter (1980), using the notation from the Table 4:

$$\widehat{Se}_1 = \frac{(g_1e_2 - e_1g_2)/n_1n_2 + a_2/n_2 - a_1/n_1 + F}{2(e_2/n_2 - e_1/n_1)},$$

$$\widehat{Se}_2 = \frac{(g_2e_1 - e_2g_1)/n_1n_2 + a_2/n_2 - a_1/n_1 + F}{2(g_2/n_2 - g_1/n_1)},$$

$$\widehat{Sp}_1 = \frac{(f_1h_2 - h_1f_2)/n_1n_2 + d_1/n_1 - d_2/n_2 + F}{2(e_2/n_2 - e_1/n_1)},$$

$$\widehat{Sp}_2 = \frac{(f_2h_1 - h_2f_1)/n_1n_2 + d_1/n_1 - d_2/n_2 + F}{2(g_2/n_2 - g_1/n_1)},$$

$$\widehat{Pr}_1 = 0.5 - \left\{\frac{[(g_1/n_1)(e_1/n_1 - e_2/n_2) + (e_1/n_1)(g_1/n_1 - g_2/n_2) + a_2/n_2 - a_1/n_1]}{2F}\right\}, \text{and}$$

$$\widehat{Pr}_2 = 0.5 - \left\{\frac{[(g_2/n_2)(e_1/n_1 - e_2/n_2) + (e_2/n_2)(g_1/n_1 - g_2/n_2) + a_2/n_2 - a_1/n_1]}{2F}\right\},$$

where

$$F = \pm\left[\left(\frac{g_1e_2 - g_2e_1}{n_1n_2} + \frac{a_1}{n_1} - \frac{a_2}{n_2}\right)^2 - 4\left(\frac{g_1}{n_1} - \frac{g_2}{n_2}\right)\frac{a_1e_2 - a_2e_1}{n_1n_2}\right]^{0.5}.$$

Some issues need to be assessed when the Hui and Walter MLEs method is used for estimation. Due to the sign of F, two sets of solutions are provided by the equations, but only one of them gives reasonable estimates assuming that Se + Sp >1. However, in some situations, there are no obvious solutions to the equations. This can occur, for instance, when more than two populations

are considered in the model, such as in the case of estimating prevalence of disease for different animal populations (herds or flocks). Other limitation is that, even when explicit formulas for the MLEs are available, the formulas for S.E. are complicated involving the calculation of the inverse of minus second derivative matrix of the log posterior evaluated at the mode. In addition, for the S.E. to be useful, for the estimation of reasonably accurate C.I. of the parameters, the sample size needs to be relatively large (Johnson, Gastwirth, & Pearson, 2001)

The Bayesian approach, which is the focus method of this section, can be used to model a priori scientific knowledge and combine this with actual information through observed data, in order to make inferences about the unknown parameters (Enoe, Geordais, & Johnson, 2000).

1.4.1 Bayesian approach for assessment of diagnostic test and disease prevalence

There are two main philosophical positions for the use of probability models for describing the real world: that probabilities are determined by the outside world (collection of data), and the other one is that probabilities are inside the people minds or opinions (current state of knowledge). Bayesian statistics combines these two positions to obtain posterior probabilities describing a particular event. It starts using probabilities to describe someone's current state of knowledge, then incorporates information through the collection of data (Christensen, Johnson, Branscum, & Hanson, 2011)

The information from the priors distributions allow excluding unrealistic and non-necessary data from the analytical environment (Christensen, Johnson, Branscum, & Hanson, 2011).

It is possible to specify prior distributions by modeling the probability of the unknown parameters using the beta distributions. One advantage of using beta priors is it simplifies the calculations and, by modifying its two parameters (a,b), it can yield a large array of shapes (Enoe, Geordais, & Johnson, 2000).

To construct beta prior distributions, it is possible to obtain information from previous similar studies or experts opinion elicitation about two or more characteristics of the distribution. First, it is necessary to elicit the most probable value or best guess (θ_0). Then, it is required to determine a lower or upper value (θ_L, θ_U), in which the expert is (1-γ/2) certain that the parameter would be larger or smaller respectively. These values become then the γ/2×100 th or (1-γ/2) ×100th percentiles of the prior distribution (e.g. if γ=0.1 then θ_L, θ_U are the 5th and 95th percentiles of the distribution). It is recommended to construct a graphical representation of the prior distributions and present it to the experts, in order to verify this distribution match with their opinion (Enoe, Geordais, & Johnson, 2000).

Calculations for sample size are not going to be described in detail in this review. For more details the reader may refers to Georgiadis, Johnson, & Gardner (2005). However, it is important to mention some statements about the sample size determination for assessment of diagnostic tests in the absence of a gold standard. Sample size calculations rely in the asymptotic normality of the MLEs of parameters. Then the sample size (*n*), for a fixed desired width of the confidence interval for the parameter of interest (*w*), has the form:

$$n = \left(2Z\,\widehat{\omega}/_{w}\right),$$

where Z is the appropriate percentile of the normal distributions and $\widehat{\omega}$ represents the estimate of the dispersion parameter (Georgiadis, Johnson, & Gardner, 2005). In practice the sample size calculation, involve the use of the guesses (modes), the Z value, and the desired widths for all the parameters of interest (Se, Sp, and Pr). The mode and width for each parameter can be extracted from the join prior distribution elicited from the experts. The sample size for a particular study of diagnostic test accuracy should be the highest *n* obtained from the calculations for each parameter (Georgiadis, Johnson, & Gardner, 2005).

After the sample size has been determined, the experimental scenario should be considered as follow: $L \geq 1$ populations are sampled independently where n_k individuals are selected from population k. $Q \geq 1$ tests are applied to each sampled individual. The data can be presented using 2^Q-contingensy tables where the observations are cross-classified according to the outcomes. Since the results of the tests are dichotomous, there are 2^Q possible outcomes (Branscum, Gardner, & Johnson, 2005; Enoe, Geordais, & Johnson, 2000).

The collected data are a set of vectors of the form $y_k = (y_{1\cdots 1k}, y_{1\cdots 2k}, \cdots, y_{2\cdots 2k})$, with correspondent cell probabilities $p_k = (p_{1\cdots 1k}, p_{1\cdots 2k}, \cdots, p_{2\cdots 2k})$. This data set is assumed to have independent multinomial distribution with the form

$$y_k \sim multinomial\big(n_k, (p_{1\cdots 1k}, p_{1\cdots 2k}, \cdots, p_{2\cdots 2k})\big),$$

where the likelihood contributions are determined as the multinomial probability of observing data in each cell, conditional on the parameters for the population k, for the case of two test is given by:

$$(T_1, T_2): p_{11k} = Pr_k Se_1 Se_2 + (1\text{-}Pr_k)(1\text{-}Sp_1)(1\text{-}Sp_2),$$

$$(T_1, \overline{T}_2): p_{12k} = Pr_k Se_1 (1\text{-}Se_2) + (1\text{-}Pr_k)(1\text{-}Sp_1) Sp_2,$$

$$(\overline{T}_1, T_2): p_{21k} = Pr_k (1\text{-}Se_1) Se_2 + (1\text{-}Pr_k) Sp_1 (1\text{-}Sp_2), \text{ and}$$

$$(\overline{T}_1, \overline{T}_2): p_{22k} = Pr_k (1\text{-}Se_1)(1\text{-}Se_2) + (1\text{-}Pr_k) Sp_1 Sp_2.$$

And the general case extended for j tests is

$$\begin{matrix} p_{1\cdots 1k} \\ p_{1\cdots 2k} \\ \vdots \\ p_{2\cdots 2k} \end{matrix} = \begin{matrix} Pr_k \times Se_1 \times \cdots \times Se_j + (1 - Pr_k) \times (1 - Sp_1) \times \cdots \times (1 - Sp_j) \\ Pr_k \times Se_1 \times \cdots \times (1 - Se_j) + (1 - Pr_k) \times (1 - Sp_1) \times \cdots \times Sp_j \\ \vdots \\ Pr_k \times (1 - Se_1) \times \cdots \times (1 - Se_j) + (1 - Pr_k) \times Sp_1 \times \cdots \times Sp_j \end{matrix}.$$

All the above considerations are based on the assumption of conditional independence of the tests (Branscum, Gardner, & Johnson, 2005; Enoe, Geordais, & Johnson, 2000).

Once the prior distribution and the ML model have been specified, the product of the likelihood function and the joint prior density are the conditional probability density of the parameters given the observed data. The unobservable (latent) data are simulated from the joint posterior distribution by an iterative Markov chain Monte Carlo (MCMC) method using the Gibbs sampler (Enoe, Geordais, & Johnson, 2000). Briefly, The Markov chain simulation method is a method for generating sequence of random vectors. This method relies on the basis that every new step only depends on the immediately previous step. In other words, what happens on step *k* only depends on what happened at step *k*-1, which means that the simulation does not depends on the history of previous iteration beyond the last step (Christensen, Johnson, Branscum, & Hanson, 2011). Near the beginning of the iterations, the sequence can take almost any value, but with every step, the distribution eventually settles down to the posterior distribution. Intuitively, observations obtained after the chain has settled down to the posterior will be more useful in estimating probabilities, thus dropping the early observations is a common practice; this is called the *Burn-in* period (Christensen, Johnson, Branscum, & Hanson, 2011). The Gibbs sampling is one of the methods used to construct the Markov chain; it is very useful since it is possible to isolate the conditional distribution of each parameter given all of the other parameters (Christensen, Johnson, Branscum, & Hanson, 2011). Gibbs sampling process has two steps: first, starting values for the parameters are selected. These values can be samples from the prior distributions, and a set of values from the latent (unobservable infected) individuals are sampled from the respective binomial distributions, which in turns are combined with the prior, resulting in independent beta posteriors for each parameter. 2) These resulting distributions are then sampled giving new values, which are used to resample the conditional binomial distribution; this process is continued until the posteriors settle down to the posterior (Enoe, Geordais, & Johnson, 2000).

If the assumptions hold, it is possible to made accurate inferences from the simulated posterior distributions. For instance, a central tendency parameter and extreme dispersion limits (e.g. estimate = mode, probability intervals (PI) = 5^{th} and 95^{th} percentiles) of the Monte Carlo sample can be accurate descriptors of the parameters of interest (Enoe, Geordais, & Johnson, 2000).

1.4.1.1 Bayesian simulation in practice

BUGS (Bayesian inference Using Gibbs Sampling) is a package of flexible software for the Bayesian analysis of statistical models using MCMC methods. In this section, I will briefly illustrate the steps for a Bayesian analysis in a simple 2 populations - 2 imperfect tests case. Following the above notation the model is specified.

$$y_{k\cdot\cdot} \sim multinomial\left(n_k, (p_{k11}, p_{k12}, p_{k21}, p_{k22})\right),$$

$$p_{k11} = Pr_k Se_1 Se_2 + (1-Pr_k)(1-Sp_1)(1-Sp_2)$$

$$p_{k12} = Pr_k Se_1 (1-Se_2) + (1-Pr_k)(1-Sp_1) Sp_2$$

$$p_{k21} = Pr_k (1-Se_1) Se_2 + (1-Pr_k) Sp_1 (1-Sp_2)$$

$$p_{k22} = Pr_k (1-Se_1)(1-Se_2) + (1-Pr_k) Sp_1 Sp_2.$$

Prior distributions for each parameter are

$$Se_1 \sim Beta\left(a_{Se_1}, b_{Se_1}\right), Se_2 \sim Beta\left(a_{Se_2}, b_{Se_2}\right), Sp_1 \sim Beta\left(a_{Sp_1}, b_{Sp_1}\right), Sp_2 \sim Beta\left(a_{Sp_2}, b_{Sp_2}\right),$$

$$Pr_k \sim Beta\left(a_{Pr_k}, b_{Pr_k}\right), Pr_k \sim Beta\left(a_{Pr_k}, b_{Pr_k}\right), k = 1,2.$$

For illustration, specification of the model and the tests results were modified from http://www.epi.ucdavis.edu/diagnostictests/index.html. This observed values are summarized in the Table 5.

Table 5. Test results states as positive (T+) or negative (T-), and prior distribution parameters for 2 population-2 tests example.

Population 1	**Test 2**		
Test 1	T+	T-	
T+	*3*	*0*	*3*
T-	*3*	*24*	*27*
	6	*24*	*30*
Population 2	Test 2		
Test 2	T+	T-	
T+	*0*	*0*	*0*
T-	*3*	*129*	*132*
	3	*129*	*132*
Prior distributions	5^{th} percentile	Mode	95^{th} percentile
Se1	-	55	85
Se2	60	90	-
Sp1	80	98	-
Sp2	60	85	-
Pr1	-	3	30
Pr2	8	30	

In the software interface window, the model is specified in braces after the word "**model**." Then the values from the data set (sample size, cell values, dimension of the tables, and number of tests) are listed in brackets after the first word "**list**." Finally, for this case illustration, initials values are set in brackets after the second "**list**" based on the modes of the priors distributions;

```
"model{
 y1[1:Q, 1:Q] ~ dmulti(p1[1:Q, 1:Q], n1)
 y2[1:Q, 1:Q] ~ dmulti(p2[1:Q, 1:Q], n2)

 p1[1,1] <- Pr1*Se1*Se2 + (1-Pr1)*(1-Sp1)*(1-Sp2)
 p1[1,2] <- Pr1*Se1*(1-Se2) + (1-Pr1)*(1-Sp1)*Sp2
 p1[2,1] <- Pr1*(1-Se1)*Se2 + (1-Pr1)*Sp1*(1-Sp2)
 p1[2,2] <- Pr1*(1-Se1)*(1-Se2) + (1-Pr1)*Sp1*Sp2
 p2[1,1] <- Pr2*Se1*Se2 + (1-Pr2)*(1-Sp1)*(1-Sp2)
 p2[1,2] <- Pr2*Se1*(1-Se2) + (1-Pr2)*(1-Sp1)*Sp2
 p2[2,1] <- Pr2*(1-Se1)*Se2 + (1-Pr2)*Sp1*(1-Sp2)
 p2[2,2] <- Pr2*(1-Se1)*(1-Se2) + (1-Pr2)*Sp1*Sp2

 Se1 ~ dbeta(2.82, 2.49)  ## Mode=0.55, 95% sure Se1 < 0.85
 Sp1 ~ dbeta(15.7, 1.30)  ## Mode=0.98, 95% sure Sp1 > 0.80
 Pr2 ~ dbeta(1.73, 2.71)  ## Mode=0.30, 95% sure Pr2 > 0.08
 Se2 ~ dbeta(8.29, 1.81)  ## Mode=0.90, 95% sure Se2 > 0.60
 Sp2 ~ dbeta(10.69, 2.71) ## Mode=0.85, 95% sure Sp2 > 0.60
 Pr1 ~ dbeta(1.27, 9.65)  ## Mode=0.03, 95% sure Pr2 < 0.30
```

```
}
list(n1=30, n2=132,
y1=structure(.Data=c(3,0,3,24),.Dim=c(2,2)),
y2=structure(.Data=c(0,0,3,129),.Dim=c(2,2)), Q=2)

list(Pr1=0.03, Pr2=0.30, Se1=0.55, Sp1=0.98, Se2=0.90,
Sp2=0.85)"
```

I. The first step is checking the model using the "specification tool."

II. If the model is syntactically correct, the data can be loaded.

III. Then the number of chains is chosen and the model can be compiled.

IV. The software requires loading the initial values. Once the initial values have been loaded, the model is initialized.

V. In order to extract the inferences for the posterior distributions, it is necessary to specify which parameters need to be monitored.

VI. The *Burn-in* period is set.

VII. The number of interactions is selected and the model is run.

VIII. Once the model have been updated, it is possible to obtain the statistics and plots for inferences and for evaluating the performance of the simulation. For this case, the obtained statistics are summarized in the Table 6.

Table 6. Summary statistics for illustration of Bayesian estimation of Se and Sp with no reference test.

	mean	**sd**	**MC_error**	**val2.5pc**	**median**	**val97.5pc**	**start**	**sample**
Pr1	0.1609	0.0645	5.80E-04	0.05577	0.1543	0.3028	1000	18002
Pr2	0.01815	0.01343	1.52E-04	0.001863	0.01514	0.05207	1000	18002
Se1	0.5207	0.163	0.001447	0.2206	0.517	0.8388	1000	18002
Se2	0.8559	0.09461	9.25E-04	0.6294	0.8743	0.9831	1000	18002
Sp1	0.9923	0.006642	7.69E-05	0.9749	0.9942	0.9996	1000	18002
Sp2	0.9633	0.01636	1.53E-04	0.9266	0.9654	0.9895	1000	18002

1.4.2 Assumptions

When the Bayesian approach is chosen as the best method for assessing diagnostic test accuracy, several assumptions need to be properly appraised. Common assumptions for the Bayesian approach are: i) the tests are conditionally independent given the true disease state, ii) the tests have the same properties in all populations, in other words, the Se and Sp between the populations is homogeneous, and iii) the tested individuals are divided into two or more populations with different disease prevalence (Johnson, Gardner, Metoyer, & Branscum, 2009; Toft, Jørgensen, & Højsgaard, 2005; Hui & Walter, 1980).

In the case of comparing tests that have similar biological basis, the lack of independence is assumed and needs to be accounted in the models (Branscum, Gardner, & Johnson, 2005). A set of tests are considered independent when the when the Se (or Sp) of the second test does not depends on whether results of the first test is positive or negative and vice versa (Gardner, Stryhn, Lind, & Collins, 2000). It is possible to express conditional dependence between Se of two tests as:

$$P(T_1 \text{ and } T_2|D) \neq P(T_1|D) \times P(T_2|D) \text{ or}$$

$$P(T_2|T_1,D) \neq P(T_2|\overline{T}_1,D) \text{ and}$$

$$P(T_1|T_2,D) \neq P(T_1|\overline{T}_2,D)$$

(Branscum, Gardner, & Johnson, 2005; Gardner, Stryhn, Lind, & Collins, 2000).

Similar expressions apply to dependence of tests specificity replacing D with $\overline{D}$. Then, the covariances between tests are given by:

$$Cov_{Se} = P(T_1 \text{ and } T_2|D) - P(T_1|D) \times P(T_2|D) \text{ and}$$

$$Cov_{Sp} = P(T_1 \text{ and } T_2|\overline{D}) - P(T_1|\overline{D}) \times P(T_2|\overline{D}).$$

These covariance between the tests outcomes satisfies

$$(Se_1 - 1)(1 - Se_2) \leq Cov_{Se} \leq min(Se_1, Se_2) - Se_1 Se_2 \text{ and}$$

$$(Sp_1 - 1)(1 - Sp_2) \leq Cov_{Sp} \leq min(Sp_1, Sp_2) - Sp_1 Sp_2$$

for infected and not infected individuals respectively (Branscum, Gardner, & Johnson, 2005; Gardner, Stryhn, Lind, & Collins, 2000). Since it is probable that prior information about the two covariances may be absent, it is logical to use uniform prior distributions over the range of the two covariances, i.e.

$$cov_{D^+} \sim uniform\big((Se_1\text{-}1)(1\text{-}Se_2), min(Se_1, Se_2)\text{-}Se_1Se_2\big) \text{ and}$$

$$cov_{D^-} \sim uniform\big((Sp_1\text{-}1)(1\text{-}Sp_2), min(Sp_1, Sp_2)\text{-}Sp_1Sp_2\big)$$

(Branscum, Gardner, & Johnson, 2005).

In addition, with this parameterization, the conditional correlations between the tests outcomes can be estimated as

$$r_{Se} = \frac{Cov_{Se}}{\sqrt{Se_1(1 - Se_1)Se_2(1 - Se_2)}} \text{ and}$$

$$r_{Sp} = \frac{Cov_{Sp}}{\sqrt{Sp_1(1 - Sp_1)Se_2(1 - Sp_2)}}$$

(Branscum, Gardner, & Johnson, 2005).

In order to take into account the conditional dependence between tests, the covariate terms need to be included in the model in each cell probability as:

$$(T_1, T_2): p_{11k} = Pr_k[Se_1Se_2 + \mathbf{Cov_{Se}}] + (1\text{-}Pr_k)\big[(1\text{-}Sp_1)(1\text{-}Sp_2) + \mathbf{Cov_{Sp}}\big]$$

$$(T_1, \overline{T}_2): p_{12k} = Pr_k[Se_1(1\text{-}Se_2) - \mathbf{Cov_{Se}}] + (1\text{-}Pr_k)\big[(1\text{-}Sp_1)Sp_2 - \mathbf{Cov_{Sp}}\big]$$

$$(\overline{T}_1, T_2): p_{21k} = Pr_k[(1\text{-}Se_1)Se_2 - \mathbf{Cov_{Se}}] + (1\text{-}Pr_k)\big[Sp_1(1\text{-}Sp_2) - \mathbf{Cov_{Sp}}\big]$$

$$(\overline{T}_1, \overline{T}_2): p_{22k} = Pr_k[(1\text{-}Se_1)(1\text{-}Se_2) + \mathbf{Cov_{Se}}] + (1\text{-}Pr_k)\big[Sp_1Sp_2 + \mathbf{Cov_{Sp}}\big]$$

Another important assumption for the Hui and Walter MLEs is the assumption that Se and Sp have to remain stable along the populations. This assumption is sometimes difficult to achieve depending on the type of population or in the splitting characteristic (Enoe, Geordais, & Johnson, 2000). If the Se or Sp are prone to change from splitting a population, in addition to the initial 3 d.f., 3 to 5 unknown parameters are being included as well (Toft, Jørgensen, & Højsgaard, 2005). For instance, a split based on biological factors such as age or sex is often not appropriate, since those factors can make that the Se (or Sp) differ from one population to the other. For example, due to change in cross-reaction related to age (Toft, Jørgensen, & Højsgaard, 2005). It is recommended, when one population can be spitted in order to give more d.f. to the model, that the covariate used for this purpose need to be independent of the tests accuracy (Toft, Jørgensen, & Højsgaard, 2005).

It is clear that if the assumption of different disease prevalence does not hold, splitting a population could increase the number of uncertainties in the model over the number of d.f. (Toft, Jørgensen, & Højsgaard, 2005). It is intuitive as well that if the populations have the same disease prevalence, the data can be treated as if they came from one population (Toft, Jørgensen, & Højsgaard, 2005).

1.4.3 Identifiability and analysis of the model

The identifiability of a Bayesian model can be described as the existence of necessary amount of information required to made valid inferences from the posterior sampling. A model can lack from identifiability if the number of unknown parameters is larger than the degrees of freedom (Toft, Jørgensen, & Højsgaard, 2005). Ideally, the inclusion of one population with different prevalence and with stable accuracy parameters, adds to the model three more d.f., in which case, to obtain accurate estimates from the posterior sample can be plausible (Toft, Jørgensen, &

Højsgaard, 2005). In the same way, when a 2 population-2 test model lacks from identifiability, the addition of the results from one conditional independent test can solve the problem (Gardner, Stryhn, Lind, & Collins, 2000). However, in most of the cases to assure these assumptions may be challenging. Here is where the researcher needs to be cautious for the construction of inferences and conclusions (Toft, Jørgensen, & Højsgaard, 2005).

The Bayesian analysis should include what is called a *sensitivity analysis*, which involves running the model with non-informative priors as well as different few perturbations of the given prior distributions. One can expect that the corresponding posterior inferences do not change drastically. If the posterior inferences are too different for the different prior distribution, this may indicate a excessive dependence of the priors (Enoe, Geordais, & Johnson, 2000).

The convergence of the Markov chains can be assessed by using sets of different starting values. It is suggested to include the best guesses of the parameters as starting values for one of the chains; also midpoints are often used as starting points of the Gibbs sampling, It is expected that the chains with different starting values settle down to the same posterior distribution (Enoe, Geordais, & Johnson, 2000; Christensen, Johnson, Branscum, & Hanson, 2011)

The assessment of the autocorrelation is used to evaluate the conditional independence of the steps of the Markov chain, since the iterative steps are executed by a software that are not necessarily random, but the samples can be thinned to give an approximation to random sampling (Christensen, Johnson, Branscum, & Hanson, 2011). It is expected that every subsequent step of the simulation to be independent of the previous steps of the chain beyond the last step (Christensen, Johnson, Branscum, & Hanson, 2011).

2 MATERIALS AND METHODS

2.1 SAMPLES

2.1.1 Source of samples

All fecal samples that were submitted to the Parasitology Section of the Colorado State University Veterinary Diagnostic Laboratory (VDL)[1] were included in the study. Most of samples exceeded 5 g to be considered eligible. All samples were tested with four diagnostic tests for the aim of detection of *Giardia* cysts and *Cryptosporidium* oocysts. All samples were stored as recommended by the test manufacturer.

2.1.2 Sample size

Sample size was estimated using $n \geq \frac{P(1-P)z_{1-\alpha/2}^2}{(Pe)^2}$, where P is the best guess of the parameter, $z_{1-\alpha/2}$ is the $(1-\alpha/2)\times 100$th percentile of the normal distribution, and e is the allowed error.

2.2 LABORATORY TESTS

2.2.1 Diagnostic tests

Test 1. MERIFLUOR® *Cryptosporidium-Giardia* is a monoclonal direct immunofluorescence detection kit for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts in fecal material (Meridian Diagnostics, Inc., Cincinnati, Ohio).

Test 2. IVD *Cryptosporidium*/*Giardia* Fecal Direct Fluorescent Antigen (DFA) detection kit is an in vitro diagnostic immunoassay for the detection of *Cryptosporidium* oocysts and *Giardia* cysts, in feces using fluorescent microscopic visualization (IDV Research Inc., Carlsbad, CA).

[1] Diagnostic Medicine Center, 300 West Drake Road, Building C, Colorado State University, Fort Collins, CO 80523-1644.

These above techniques utilize the principle of direct fluorescence antigen (DFA) detection. The detection reagent contains a mixture of Fluorescein-isothiocyanate (FITC) labeled monoclonal antibodies directed against the parasite's oocyst/cyst wall antigens. The specimens are place on treated slide and are treated with the detection reagent and counter stain. If present, the antibodies attach to cells; the slides are rinsed to remove the unbound antibodies. The slide is examined under a fluorescent microscope looking for the specific morphologic characteristics.

Test 3. IVD® *Giardia* Antigen Detection Microwell ELISA is an *in vitro* immunoassay for the determination of *Giardia* antigen in fecal samples (IDV Research Inc., Carlsbad, CA). If present, the antigen is captured by antibodies attached to the microwells. The wells are incubated and washed before anti-*Giardia* antibodies conjugated to peroxidase are added. After washed to remove unbound enzyme complex, a chromogen is added which change color if the enzyme complex is present. Finally, a stop solution is added to stabilize the reaction. The results can be obtained by comparing to a visual scale or through colorimetric spectrophotometry.

Test 4. IDEXX Snap *Giardia* test kit is a rapid ELISA for the detection of *Giardia* antigen in canine and feline feces (IDEXX Laboratories Inc., Westbrook, ME). Each sample is diluted and mixed with the conjugate containing labeled antibodies. If antigen is present, an antigen/antibody complex is formed. The suspension of antigen/antibody complex is added to the sample well, the sample flows laterally throughout a membrane. The antigen/antibody complexes then react with the other reagents. The results are obtained by the presence or absence of visual reaction spots in the output window of the device.

2.2.2 Sample processing

All samples were stored and prepared according to the manual indications (and to the Standard Operating procedure for MeriFluor procedure). Samples to be analyzed with Test-1 and Test-2

were refrigerated at 2 to 8°C for up to 7 days, concentrated, stained, and read in parallel. Samples for the Test-3 and Test-4 were frozen at -40° and thawed once for processing.

2.2.2.1 Concentration of samples for DFA

I. 3g (always > 1g) of sample was weighed and diluted at ratio of 1:1.5 with PBS-EDTA buffer solution.

II. The sample was homogenized and strained through 2 layers of sterile gauze.

III. The liquid was layered carefully on top of 7ml of gradient sugar solution (1.13 sp.g) in a 15ml centrifuge tube.

IV. The tubes were centrifuged at 800 x g for 10min in a fixed angle centrifuge.

V. Without disturbing the liquid-sugar interface, the liquid and debris was pipetted off the interface, and placed in a second 15ml centrifuge tube.

VI. 7.5ml of PBS-EDTA buffer was added to the new tube, and then the sample was homogenized and centrifuged at 1200 x g for 10 min.

VII. The supernatant was discarded and the VI step was repeated.

VIII. After the last centrifugation, the supernatant was discarded and the pellet was re-suspended to 1ml with PBS-EDTA. This contains a concentrated suspension of oocysts and cysts.

This concentrated suspension was storage at 2 to 8°C for up to 3 weeks.

2.2.2.2 Staining and examination of samples with DFA

The kit contains treated slides, transfer loops, mounting media, conjugate, counter stain, wash buffer, and positive and negative control samples. A single set of controls were used for each batch of samples (10-20).

I. A drop of the positive and negative control samples were transferred to proper identified slides.

II. 15µl of each homogenized concentrated sample were transferred to each identified well. This was done in a random way, by a different technician, within the two kits used, in order to minimize classification bias.

III. After the samples have air dried at room temperature, a drop of detection reagent, and a drop of counter stain were added.

IV. The samples were incubated for 30min in a dark humidity chamber.

V. Then, the slides were gently stream rinsed with the wash buffer. The slides were tip by the long side in a paper tower to eliminate the excess of fluid.

VI. A drop of mounting media was added and a cover slide was placed.

VII. Finally, the slides were examined under the fluorescent microscopy (excitation wave-length 490-500, barrier filter 510-530).

Interpretation of DFA

The professional crew of the parasitology laboratory trained the reader for the microscopic identification of oocysts. This training consisted in reading of batches of samples slides previously stained with Test 1 kit. The obtained results were compared with the official results assigned to those samples. The training was done until the required agreement was reached (perfect agreement for each batch). In necessary to mention that, since the reader was in training while the samples were being collected and processed, the professional technician trainer also checked the first 20% of the samples and those results were included in the study.

A sample was declared as *Giardia* positive if the technician observed ovals cyst, 8-12µm long, which stains bright apple-green. The count of cysts was recorded Figure 5.

A sample was declared as *Cryptosporidium* positive if the technician observed round oocyst, 2-6µm diameter, which stains bright apple-green Figure 5.

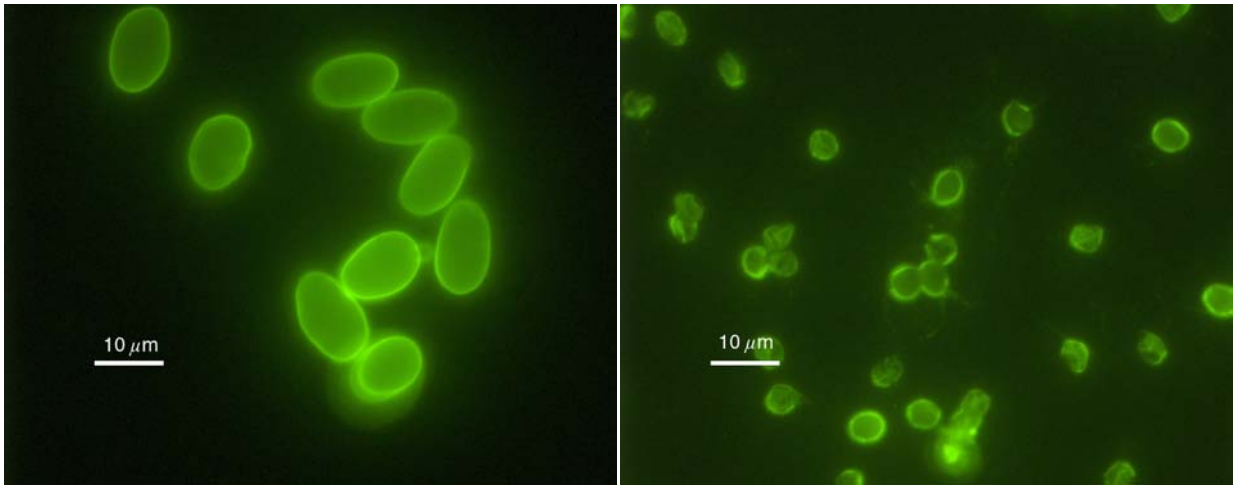


Figure 5. *Giardia* cysts (left) and *Cryptosporidium* oocysts (right) under the fluorescent microscope.

2.2.2.3 Test-3 procedure

Components of the kit are microwells containing anti-*Giardia* antibodies, enzyme conjugate, positive control, negative control, chromogen, wash solution, dilution buffer, and stop solution. All the reagents and samples were at room temperature (15-25°C) before starting the process.

I. Approximately 0.1g of sample was diluted in 0.7ml of dilution buffer.

II. 100µl of negative and 100µl of positive control were transferred to an identified well respectively.

III. 100µl of each diluted sample was added to a well, followed by 60 minutes incubation at room temperature.

IV. The wells were thoroughly washed with wash buffer and slapped in a paper towel to eliminate de excess of fluid.

V. 2 drops of enzyme conjugate were added to each well, followed by 30min incubation.

VI. Step IV was repeated.

VII. 2 drops of chromogen was added to each well, followed by 10min incubation.

VIII. 2 drops of stop solution was added to each well. The wells were mixed by gently tapping the wells for at least 15 seconds.

IX. The reaction was read with spectrophotometer within 5 minutes of adding the stop solution.

Interpretation of Test-3

The wells were read using a dichromatic reading with filters at 450nm and 630nm. As defined by the manufacturer, the sample was declared as positive if the absorbance was at least 0.08 OD and above. The sample was declared as negative if the absorbance was less than 0.08 OD.

2.2.2.4 Test-4 test procedure.

The kit components are conjugate/swabs, each conjugate/swab contains 0.7 ml of anti-*Giardia* : peroxidase conjugate solutions, each Test-4 device contains 0.4ml of wash solution and 0.6 ml of substrate solution.

All the reagents were at room temperature and samples were thawed before starting the procedure.

I. The tip of the conjugate/swab was coated with a thin layer of sample.

II. With the cover tube placed back over the swab, the plastic valve was broken to allow the conjugate to dilute the sample by squeezing and releasing the bulb three times.

III. With the Test-4 device in a flat surface, 5 drops of the conjugate-sample solution was dispensed in the sample well.

IV. After the sample had flowed completely across the result window, the Test-4 device was activated.

V. The results were read at 8 minutes.

Interpretation of Test-4

A sample was declared as negative if there was no color on the sample spot and in the negative control spot, or when color on the sample spot is equal to the color on the negative control spot.

A sample was declared as positive if the color in the sample spot was darker than the color on the negative control spot.

2.2.2.5 Additional information

Additional information as species, age (months), sex (male/female), origin of the sample (VTH/outside), reason for submission were recorded with the aim of choosing a covariate to split the population. The texture of the samples was also recorded using a fecal scoring system[2] (Figure 60).

Figure 6. Nestlé PURINA fecal scoring system.

2.3 BAYESIAN STATISTICAL ANALYSIS

[2] Purina® Fecal Scoring System for Dogs and Cats, Nestlé-Purina Pet Food Co, St Louis, MO.

2.3.1 Prior distribution elicitations

The elicitation of the prior distribution was done trough a short survey (0) that was submitted to three experts. The experts were chosen by Dr. Lora Ballweber head of the parasitology laboratory. This survey contains a list of the four tests followed by a graphical example and a text explanation of that graphic. The questions were written in a way that the terms sensitivity, specificity, and prevalence were substituted by concepts related to known infected samples, known non-infected samples, and proportion of infected individuals respectively, in order to avoid over-thinking regarding the concepts meaning. The experts were asked to give the mode and the 5th percentile for the construction of the Se and Sp prior distribution, and the mode and 95th percentile for the Pr prior distribution. Experts were asked to not answer the questions if they felt they did not have not enough experience or expertise in a particular test or parasite. Beta Buster software by Chun-Lung Su freeware available from http://www.epi.ucdavis.edu/diagnostictests/betabuster.html was used to obtain the *a* and *b* parameters of the beta priors.

2.3.2 Assumptions and considerations

An attempt was made to get more information about the specific antibody used for the four tests, but as expected, this information was denied since this kind of information is part of the confidential property of the manufacturing companies. In this case, it is logical to assume that, since the tests have the same biological principle of detection of antigen through the bond with a monoclonal antibody, they have certain degree of conditional dependence at least in their specificity. In the case of sensitivity, an assumption was made that the two DFA tests have conditional dependence, since the positive samples are determined by the same criteria. In the same way, it was

assumed that Test-3 and Test-4 tests sensitivities are conditional dependent, since both test use colorimetric scales to declare the positive samples.

It was decided to use the texture of the sample as the splitting covariable, because, according to experts and to the manufactures' instructions, the texture of the sample does not affect the performance of the tests. It was therefore assumed that Se and Sp of the tests are stable across populations. The prevalence of *Giardia* and *Cryptosporidium* was considered different between diarrheic and non-diarrheic populations.

2.3.3 Specification of the models

A. For tests with the aim of detecting *Cryptosporidium*, we have two conditional dependent tests Test-1 and Test-2, and two populations.

For this scenario, the data set has an independent multinomial distribution for each population:

$y_{1\cdot\cdot} \sim multinomial\big(n_1, (p_{111}, p_{112}, p_{121}, p_{122})\big), y_{2\cdot\cdot} \sim multinomial\big(n_2, (p_{211}, p_{212}, p_{221}, p_{222})\big),$

and cell probabilities for each *k* population

$$(T_1, T_2)\colon p_{k11} = Pr_k[Se_1 Se_2 + \boldsymbol{Cov_{Se}}] + (1 - Pr_k)\big[(1 - Sp_1)(1 - Sp_2) + \boldsymbol{Cov_{Sp}}\big]$$

$$(T_1, \bar{T}_2)\colon p_{k12} = Pr_k[Se_1(1 - Se_2) - \boldsymbol{Cov_{Se}}] + (1 - Pr_k)\big[(1 - Sp_1)Sp_2 - \boldsymbol{Cov_{Sp}}\big]$$

$$(\bar{T}_1, T_2)\colon p_{k21} = Pr_k[(1 - Se_1)Se_2 - \boldsymbol{Cov_{Se}}] + (1 - Pr_k)\big[Sp_1(1 - Sp_2) - \boldsymbol{Cov_{Sp}}\big]$$

$$(\bar{T}_1, \bar{T}_2)\colon p_{k22} = Pr_k[(1 - Se_1)(1 - Se_2) + \boldsymbol{Cov_{Se}}] + (1 - Pr_k)\big[Sp_1 Sp_2 + \boldsymbol{Cov_{Sp}}\big].$$

The prior distributions are

$$Se_1 \sim Beta\big(a_{Se_1}, b_{Se_1}\big),\ Sp_1 \sim Beta\big(a_{Sp_1}, b_{Sp_1}\big), Se_2 \sim Beta\big(a_{Se_2}, b_{Se_2}\big), Sp_2 \sim Beta\big(a_{Sp_2}, b_{Sp_2}\big),$$

$$Pr_1 \sim Beta\big(a_{Pr_1}, b_{Pr_1}\big), Pr_2 \sim Beta\big(a_{Pr_2}, b_{Pr_2}\big), \text{and}$$

$$cov_{\mathrm{Se}} \sim uniform\big((Se_1 - 1)(1 - Se_2), min(Se_1, Se_2) - Se_1 Se_2\big);$$

$$cov_{\mathrm{Sp}} \sim uniform\big((Sp_1 - 1)(1 - Sp_2), min(Sp_1, Sp_2) - Sp_1 Sp_2\big).$$

B. For test with the aim of detecting *Giardia* , we have all four tests are conditional dependent in their Sp since they share similar biological principle in detecting true negative samples. In addition to this we can assume that Test-1 with Test-2 and Test-3 with Test-4 have a conditional dependence in their Se.

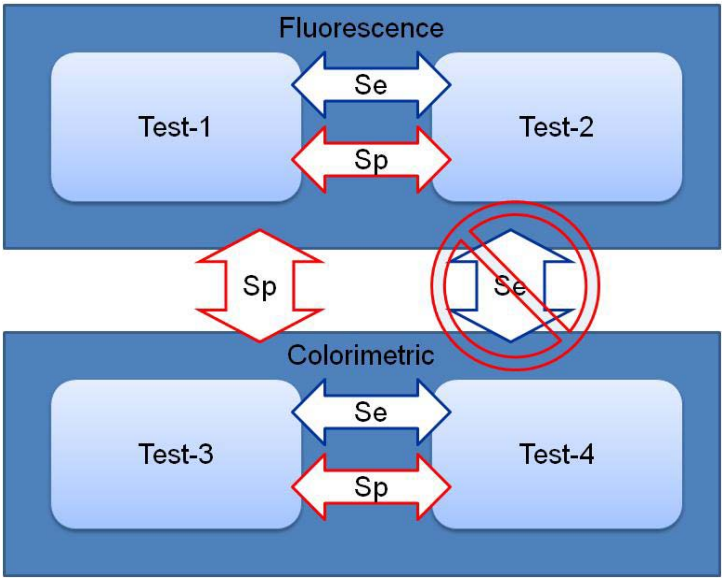


Figure 7. Schematic representation of conditional dependence of Se and Sp.

According to our assumptions, the best way to include each test in the models is by performing a pair-wise comparison, specifying models for each pair of tests. According to this, when Test-1 and Test-2 (or Test-3 and Test-4) were included, the two models are identically specified to the above scenario A. In the other hand, when a fluorescence test and a colorimetric based test were included, the covariance term for the Se was excluded; e.g., the cell probabilities for the model including Test-1 and Test-3 are:

$$(T_1, T_3): p_{k11} = Pr_k Se_1 Se_3 + (1 - Pr_k)\left[(1 - Sp_1)(1 - Sp_3) + \boldsymbol{Cov_{Sp1}}\right]$$

$$(T_1, \bar{T}_3): p_{k13} = Pr_k Se_1 (1 - Se_1) + (1 - Pr_k)\left[(1 - Sp_1) Sp_3 - \boldsymbol{Cov_{Sp1}}\right]$$

$$(\bar{T}_1, T_3): p_{k31} = Pr_k (1 - Se_1) Se_3 + (1 - Pr_k)\left[Sp_1 (1 - Sp_3) - \boldsymbol{Cov_{Sp1}}\right]$$

$$(\bar{T}_1, \bar{T}_3): p_{k33} = Pr_k (1 - Se_1)(1 - Se_3) + (1 - Pr_k)\left[Sp_1 Sp_3 + \boldsymbol{Cov_{Sp1}}\right]$$

As an attempt to increase the identifiability of the Se inferences, it was decided to specify models with three tests, including one test with no Se covariance term to the two tests models; i.e. For the model including the two fluorescence one of the colorimetric test was included, and vice versa.

For the models with three tests, it is necessary to include a three Sp covariance terms. Multinomial distributions would include one more dimension

$$y_{1..} \sim multinomial\left(n_1, \left(p_{1111}, p_{1112}, p_{1121}, p_{1122}, p_{1211}, p_{1212}, p_{1221}, p_{1222}\right)\right) \text{ and}$$

$$y_{2..} \sim multinomial\left(n_2, \left(p_{2111}, p_{2112}, p_{2121}, p_{2122}, p_{2211}, p_{2212}, p_{2221}, p_{2222}\right)\right).$$

The probability of the first cell is

$$(T_1, T_2, T_3): p_{k111}$$

$$= Pr_k \times [Se_1 Se_2 + \boldsymbol{Cov_{Se}}] \times Se_3 + \left[(1 - Sp_1)(1 - Sp_2) + \boldsymbol{Cov_{Sp1}}\right]$$

$$\times \left[\frac{(1 - Sp_1)(1 - Sp_3) + \boldsymbol{Cov_{Sp2}}}{(1 - Sp_1)}\right] \times \left[\frac{(1 - Sp_2)(1 - Sp_3) + \boldsymbol{Cov_{Sp3}}}{(1 - Sp_2)(1 - Sp_3)}\right].$$

2.3.4 Analysis of performance of models

A sensitivity analysis was done as follows: all models were run using each prior from each expert. In addition, a model was run using a weighted consensus prior distribution. This consensus distribution can be represented as follow for *i tests* and *j* experts.

$$\theta_{..} = \frac{\sum \theta_{ij}}{n_j},$$

were each θ_{ij} are the beta prior distribution obtained from the experts,

$$\theta_{ij} \sim Beta\left(a_{\theta_{ij}}, b_{\theta_{ij}}\right).$$

When an expert answered he/she had no experience with the test, the non-informative prior distributions $\theta \sim Beta(1,1)$ were included.

Other type of analysis for the performance of the models is the comparison of the inferences between the different estimates; this is called a sensitivity analysis. It is expected that the parameters do not largely differ when they are obtain from different models.

The convergence of the Markov chains was assessed by using three sets of starting values for the Gibbs sampling, which included extreme low, extreme high, and midpoint values. The convergence using the Brooks-Gelman-Rubin test (BGR) included in the WinBUGS "Sample monitor tool" was examined.

Autocorrelation of the models was assessed by analyzing the autocorrelation plots provided with the "Sample monitor tool" in the WinBUGS software.

3 RESULTS

3.1 LABORATORY TESTS

A total of 201 samples were collected and processed over a time period of 18 weeks. Table 7 depicts the results of each test when applied to all samples. When the aim is detecting *Giardia,* the apparent prevalence detected does not differ greatly between the four tests. In contrast, the APr (apparent prevalence) of Test-2 for detection of *Cryptosporidium* is much lower than the APr of Test-1 test.

3.1.1 Ease of use of the kits.

In this section, we discuss some characteristics of the test that can utterly affect the results and the analysis of the study.

3.1.1.1 Time of performance

DFA tests (Test-1 and Test-2) are the longest in time of performance. Sample concentrations may last an average of 45 minutes depending on the number of samples. The preparation of slides, including the incubation times and mounting of slides, can last between 30 to 45 minutes depending, also, in the number of samples. Finally, each slide of three wells containing samples can take up to 15 minutes, depending on the concentration of cysts or oocysts. In total, depending on the number of samples, the obtainment of results can take up to 3 hours.

Test-3 is a microwell ELISA test that includes several cycles of incubations and washings. Thawing the samples and getting all reagents at room temperature took approximately 30 minutes. The procedure including the dilution of samples, the incubation periods, and the time of reading took not less than 2 hours. In average, a result from a frozen sample can take from 2 and a half to 3 hours to be obtained. Test-4 is the quickest. Similar than for Test-3 thawing the sam-

ple may take up to 30 minutes. Nevertheless, once the sample is thawed, the procedure for obtaining the results takes just 8 minutes. Other notable difference between Test-1 and Test-2 is that Test-2 mountings presented a brighter background when compared to Test-1 preparations.

3.1.1.2 Detected issues during the procedures

One of the most common problems found performing the DFA tests, was that some samples did not adhere firmly to the pretreated slides. Both kits, Test-1 and Test-2 presented this problem, however, the adherence problem was more frequent in the Test-2. Other notable feature that were different between test one and two is that, in Test-2 mounted samples , the debris and residual fecal material was brighter than in Test-1 preparations.

3.2 PRIOR DISTRIBUTIONS

The prior distributions of sensitivities obtained from three experts are presented in the 0. Expert 1 gave high values of sensitivity for DFA techniques with no difference between the detection of *Giardia* or *Cryptosporidium*. However, this expert gave different values between DFA and Test-3 techniques, giving the latter a lower sensitivity. Expert 2 gave different values of sensitivity for Test-1 when used for detection of different organisms. This expert gave equal maximum values for Test-4 and Test-1 (only *Giardia*), but different widths. In general, expert 3 gave lower values of sensitivity than other experts did.

The prior distributions of specificities obtained from three experts are presented in 0. In general, all experts gave higher specificity with narrower confidence when compared to sensitivity values. Only expert one gave values for all tests.

The prior distributions of prevalence obtained from three experts are presented in 0. Expert 1 believes that prevalence of *Giardia* and *Cryptosporidium* is 0, differing only in the expert's confidence about the parameter. For expert 2, values of prevalence of *Giardia* and *Cryptosporidium*

are different; 10 and 5 for non-diarrheic samples, and 5 and 2 for diarrheic samples. With those values, we can also observe a difference between diarrheic and non-diarrheic type of sample. Expert 3 believes that prevalence of *Giardia* and *Cryptosporidium* are stable across different samples but differ in their confidence about values.

3.3 POSTERIOR DISTRIBUTIONS INFERENCES

We obtained 168 estimated values from 28 Bayesian simulation models. This makes difficult to follow the results discussion. For better understanding, each model has three unique components as inputs (Two tests results and a prior distribution) and six outputs (posterior distributions of Se and Sp for two different tests and prevalence for two populations). For estimation of parameters of tests detecting *Cryptosporidium,* we have four models, which inputs are Test 1 and Test 2 results, with each expert prior distribution (including the consensus). For tests detecting *Giardia*, it gets more complicated, since we have combinations of four tests plus the prior distributions from each expert (including the consensus). We identify each model with a composed code based on the model input. The first two numeric characters indicate the tests results used and the third alphanumeric character indicates which expert prior-distribution was used; e.g. the model identified as 1-2-E1 is a model which inputs are Test 1 and Tests 2 results plus the prior distribution elicited from expert 1. In the same way, the inputs for the model 3-4-C are results from Test 3 and Test 4 and the consensus prior distribution.

Since the number of positive samples was limited, the narrow prior distributions exert too much effect to the posterior distribution. Thus, it was decided to increase the width of the prior distribution to match the number of positive samples; this gives adequate balance to the observed data and the prior distributions.

3.3.1 Test 1 and Test 2 estimates for *Cryptosporidium* detection

0 presents the inferred statistics from the posterior distributions. Prevalence 1 refers to the prevalence of *Cryptosporidium* in non-diarrheic samples, and prevalence 2 refers to prevalence of *Cryptosporidium* in loose or diarrheic samples. Prevalence 1estimates was relatively constant across models with different priors. In the same manner prevalence, 2 estimates were constant, except for the model with expert 2 priors. The inferred values of Test-1 sensitivity were similar when with expert 2, 3, and consensus priors in the models. The values of sensitivity of Test-2 were low when compared with sensitivity of Test-1. As expected, the values of specificity were high for both tests.

3.3.2 Estimates of Se and Sp for tests detecting *Giardia*

All estimates of sensitivity and specificity calculated are presented in Table 13.

3.3.2.1 Test 1 estimates

The inferences about sensitivity were relatively stable across models with different prior-distributions. Estimated values of sensitivity, from those models with prior consensus, have narrower Probability Intervals (PI) than all other models except model 1-3-E1(Figure 8). All estimates of specificity were similar and stable across models (Figure 9).

3.3.3 Test-2 for detection of *Giardia*

When the model contains either Test 3 or Test 4, the estimated PIs for sensitivity of Test 2 were narrower than other models except estimates from model 1-2-E1 (Figure 10). The model 2-3-C was the model that provides the narrower PIs (Figure 10). In general, estimates of Se were stable from different models Inferences of Specificity of Test-2 were relatively stable across all models (Figure 11).

3.3.4 Test-3 test for detection of *Giardia*

Inferences made from the models with expert 1 prior-distributions were higher and narrower than estimates from other models except model 3-4-C (Figure 12). In general, the estimates of Test-3 sensitivity were low. All estimates of sensitivity containing informative priors were similar and stable across models. Inferences about Test-4 specificity were high and stable across models (Figure 13).

3.3.5 Test-4 test for detection of *Giardia*

The sensitivity of Test-4 was relatively stable across models. The posterior distributions from models with consensus prior-distribution were narrower (Figure 14). Estimates of Test-4 specificity were high and stable (Figure 15). Prevalence of *Giardia*

Inferred values for the estimation of Prevalence of *Giardia* are in Table 14. The inferences of prevalence, for the non-diarrheic population, were stable across models. The main variations were found for models with different prior-distributions. The models using of consensus prior-distributions gave more stable and narrower estimates than other models. For the case of the population with diarrheic samples, the prevalence was higher and stable across models.(Figure 16).

3.4 PERFORMANCE OF THE MODELS

3.4.1 Convergence

All models were run 13.000 iteration with a burn-in period of 3000. Based on BGR diagnostics, trace plots, and history plots, all models reached convergence. All models reached convergence before the iteration number 3000. However, the models with more than 2 tests were slower to reach convergence. In addition, when the initial values were too extreme, the simulation of values was unstable.

3.4.2 Autocorrelation

Lower values of area under the correlation plot indicate lower autocorrelation. For the model of Test-1 and Test-2 with the aim of detection of *Cryptosporidium,* the autocorrelation for estimated values of sensitivity and specificity were higher than autocorrelation for prevalence (0). In the model with expert 1 prior-distribution, the total autocorrelation was higher than models with other priors.

Regarding performance of the models with results of tests detecting *Giardia,* the best models for the inference of each test parameters were those with lower autocorrelation values. 0In general, for the models using detection of *Giardia,* there were no large variations in autocorrelation between models with different prior-distributions for estimation of sensitivity or specificity. Models with more than 2 tests gave high values of autocorrelation (0).In general, autocorrelation was higher for specificity than for sensitivity estimations.

Similar than in the model with *Cryptosporidium* results, the values of autocorrelation for prevalence of *Giardia* were low (0).

3.4.3 Tables and figures

Table 7. Tests results of four diagnostic tests for the detection of *Giardia*.

Parasite	**Test**	**Positive**	**Negative**	**Apparent prevalence**	**Total**
Giardia	Test-1	21	180	0.10	201
	Test-2	22	179	0.11	201
	Test-3	17	184	0.08	201
	Test-4	17	184	0.08	201
Cryptosporidium	Test-1	19	182	0.09	201
	Test-2	8	193	0.04	201

Table 8. Elicited values of sensitivity from three experts (lower confidence 5th percentile and mode).

		Expert 1		Expert 2		Expert 3	
Parasite	**Tests**	**5th perc.**	**Mode**	**5th perc.**	**Mode**	**5th perc.**	**Mode**
Giardia	Test-1	95	100	96	100	84	94
	Test-2	95	100	-	-	-	-
	Test-3	90	95	-	-	-	-
	Test-4	75	85	80	100	75	90
Crypto	TEST-1	95	100	70	85	80	90
	Test-2	95	100	-	-	-	-

Table 9. Elicited values of specificity from three experts (minimum confidence 5th percentile and mode).

		Expert 1		Expert 2		Expert 3	
Parasite	**Tests**	**5th perc.**	**Mode**	**5th perc.**	**Mode**	**5th perc.**	**Mode**
Giardia	Test-1	97	100	100	100	90	98
	Test-2	97	100	-	-	-	-
	Test-3	95	98	-	-	-	-
	Test-4	97	100	100	100	85	95
Crypto.	Test-1	97	100	100	100		
	Test-2	97	100	-	-	-	-

Table 10. Elicited values of prevalence of *Giardia* and *Cryptosporidium* from three experts. Comparison according to consistence of the sample (mode and maximum confidence 95th percentile).

		Expert 1		Expert 2		Expert 3	
		Mode	**95th perc.**	**Mode**	**95th perc.**	**Mode**	**95th perc.**
Loose or diarrheic sample	*Giardia*	0	18	10	20	15	30
	Cryptosporidium	0	15	5	10	3	7
Normal or non-diarrheic sample	*Giardia*	0	5	5	10	15	20
	Cryptosporidium	0	7	2	3	3	5

Table 11. Estimates of Se and Sp for Test-1 and Test-2 when detecting *Cryptosporidium* (Median and 95%PI).

Inputs				Estimates				
Model Code	**Test A**	**Test B**	**Prior Distribution**	**Test**	**Parameter**	**LPI (5th perc.)**	**Median**	**UPI (95th perc.)**
1-2-C	Test-1	Test-2	C	Test-1	Se	78.6	87.7	94.3
					Sp	94.7	97.3	99.5
				Test-2	Se	37.2	68.0	98.7
					Sp	97.2	99.1	99.9
1-2-E1	Test-1	Test-2	E1	Test-1	Se	39.3	78.2	99.4
					Sp	96.8	98.8	99.8
				Test-2	Se	80.5	95.9	99.8
					Sp	92.3	95.7	99.7
1-2-E2	Test-1	Test-2	E2	Test-1	Se	4.7	28.4	59.8
					Sp	94.8	97.8	99.5
				Test-2	Se	71.5	87.2	96.3
					Sp	95.8	98.6	99.9
1-2-E3	Test-1	Test-2	E3	Test-1	Se	2.9	44.8	96.1
					Sp	93.4	97.2	99.5
				Test-2	Se	69.0	87.2	97.0
					Sp	88.1	92.9	97.1

Table 12. Estimates of prevalence of *Cryptosporidium* (Median and 95%PI).

Population	**Model code**	**LPI (5th perc.)**	**Median**	**UPI (95th prec.)**
Not-diarrheic	1-2-C	1.5	2.6	5.1
	1-2-E1	0.2	2.0	6.5
	1-2-E2	1.4	2.3	3.5
	1-2-E3	1.6	3.0	4.9
Diarrheic	1-2-C	2.9	6.1	12.0
	1-2-E1	1.1	5.8	15.6
	1-2-E2	4.6	8.7	13.7
	1-2-E3	1.6	4.3	8.7

Table 13. Estimates of Sensitivity and Specificity for tests detecting *Giardia*

Inputs				**Estimates**				
Model ID	**Test A**	**Test B**	**Prior Distribution**	**Test**	**Parameter**	**LPI (5th perc.)**	**Median**	**UPI (95th perc.)**
1-2-C	Test-1	Test-2	C	Test-1	Se	87.2	94.1	98.1
1-2-C	Test-1	Test-2	C	Test-1	Sp	96.0	98.4	99.5
1-2-C	Test-1	Test-2	C	Test-2	Se	61.0	79.8	93.9
1-2-C	Test-1	Test-2	C	Test-2	Sp	92.6	96.1	98.4
1-2-E1	Test-1	Test-2	E1	Test-1	Se	87.1	97.4	99.9
1-2-E1	Test-1	Test-2	E1	Test-1	Sp	96.3	98.6	99.8
1-2-E1	Test-1	Test-2	E1	Test-2	Se	85.5	96.6	99.9

	Inputs			Estimates				
Model ID	**Test A**	**Test B**	**Prior Distribution**	**Test**	**Para-meter**	**LPI (5th perc.)**	**Median**	**UPI (95th perc.)**
1-2-E1	Test-1	Test-2	E1	Test-2	Sp	95.7	98.0	99.5
1-2-E2	Test-1	Test-2	E2	Test-1	Se	86.4	97.3	99.9
1-2-E2	Test-1	Test-2	E2	Test-1	Sp	96.6	99.1	100.0
1-2-E2	Test-1	Test-2	E2	Test-2	Se	65.3	85.8	98.8
1-2-E2	Test-1	Test-2	E2	Test-2	Sp	93.6	97.0	99.0
1-2-E3	Test-1	Test-2	E3	Test-1	Se	72.1	88.7	97.9
1-2-E3	Test-1	Test-2	E3	Test-1	Sp	95.1	98.3	99.7
1-2-E3	Test-1	Test-2	E3	Test-2	Se	60.9	83.4	98.6
1-2-E3	Test-1	Test-2	E3	Test-2	Sp	93.5	97.4	99.7
1-3-C	Test-1	Test-3	C	Test-1	Se	86.9	93.9	98.1
1-3-C	Test-1	Test-3	C	Test-1	Sp	95.6	98.2	99.5
1-3-C	Test-1	Test-3	C	Test-3	Se	68.7	86.0	97.6
1-3-C	Test-1	Test-3	C	Test-3	Sp	96.2	98.2	99.4
1-3-E1	Test-1	Test-3	E1	Test-1	Se	92.7	98.5	99.9
1-3-E1	Test-1	Test-3	E1	Test-1	Sp	94.2	97.1	99.1
1-3-E1	Test-1	Test-3	E1	Test-3	Se	88.1	93.9	97.5
1-3-E1	Test-1	Test-3	E1	Test-3	Sp	95.7	97.8	99.2
1-3-E2	Test-1	Test-3	E2	Test-1	Se	83.4	95.7	99.8
1-3-E2	Test-1	Test-3	E2	Test-1	Sp	97.6	99.5	100.0
1-3-E2	Test-1	Test-3	E2	Test-3	Se	45.7	67.7	88.1
1-3-E2	Test-1	Test-3	E2	Test-3	Sp	95.4	98.3	99.9
1-3-E3	Test-1	Test-3	E3	Test-1	Se	74.1	88.5	97.4
1-3-E3	Test-1	Test-3	E3	Test-1	Sp	94.8	98.2	99.7
1-3-E3	Test-1	Test-3	E3	Test-3	Se	44.0	68.7	92.2
1-3-E3	Test-1	Test-3	E3	Test-3	Sp	95.0	98.6	99.9
1-4-C	Test-1	Test-4	C	Test-1	Se	86.7	93.6	97.8
1-4-C	Test-1	Test-4	C	Test-1	Sp	95.5	97.9	99.4
1-4-C	Test-1	Test-4	C	Test-4	Se	74.6	84.9	92.8
1-4-C	Test-1	Test-4	C	Test-4	Sp	96.0	98.0	99.3
1-4-E1	Test-1	Test-4	E1	Test-1	Se	80.4	93.2	99.6
1-4-E1	Test-1	Test-4	E1	Test-1	Sp	95.6	98.3	99.9
1-4-E1	Test-1	Test-4	E1	Test-4	Se	63.5	80.6	93.4
1-4-E1	Test-1	Test-4	E1	Test-4	Sp	96.9	99.2	100.0
1-4-E2	Test-1	Test-4	E2	Test-1	Se	80.4	92.5	99.3
1-4-E2	Test-1	Test-4	E2	Test-1	Sp	96.9	99.0	100.0
1-4-E2	Test-1	Test-4	E2	Test-4	Se	67.1	85.1	98.7
1-4-E2	Test-1	Test-4	E2	Test-4	Sp	98.2	99.6	100.0
1-4-E3	Test-1	Test-4	E3	Test-1	Se	76.1	90.0	98.0
1-4-E3	Test-1	Test-4	E3	Test-1	Sp	94.0	97.6	99.6
1-4-E3	Test-1	Test-4	E3	Test-4	Se	62.2	79.4	93.7
1-4-E3	Test-1	Test-4	E3	Test-4	Sp	94.6	97.7	99.4
2-3-C	Test-2	Test-3	C	Test-2	Se	87.2	94.1	98.1
2-3-C	Test-2	Test-3	C	Test-2	Sp	95.3	97.9	99.4
2-3-C	Test-2	Test-3	C	Test-3	Se	42.4	62.7	83.1
2-3-C	Test-2	Test-3	C	Test-3	Sp	92.8	96.3	98.5
2-3-E1	Test-2	Test-3	E1	Test-2	Se	83.0	95.8	99.8
2-3-E1	Test-2	Test-3	E1	Test-2	Sp	93.5	96.4	98.7
2-3-E1	Test-2	Test-3	E1	Test-3	Se	73.9	91.2	98.8

	Inputs			Estimates				
Model ID	**Test A**	**Test B**	**Prior Distribution**	**Test**	**Para-meter**	**LPI (5th perc.)**	**Median**	**UPI (95th perc.)**
2-3-E1	Test-2	Test-3	E1	Test-3	Sp	95.5	97.7	99.1
2-3-E2	Test-2	Test-3	E2	Test-2	Se	84.2	96.5	99.9
2-3-E2	Test-2	Test-3	E2	Test-2	Sp	97.5	99.5	100.0
2-3-E2	Test-2	Test-3	E2	Test-3	Se	38.7	60.8	83.8
2-3-E2	Test-2	Test-3	E2	Test-3	Sp	94.4	97.5	99.6
2-3-E3	Test-2	Test-3	E3	Test-2	Se	71.9	87.6	97.5
2-3-E3	Test-2	Test-3	E3	Test-2	Sp	94.1	97.9	99.7
2-3-E3	Test-2	Test-3	E3	Test-3	Se	35.8	61.8	88.9
2-3-E3	Test-2	Test-3	E3	Test-3	Sp	93.9	98.0	99.9
2-4-C	Test-2	Test-4	C	Test-4	Se	74.3	85.0	93.1
2-4-C	Test-2	Test-4	C	Test-4	Sp	95.6	97.9	99.2
2-4-C2	Test-2	Test-4	C	Test-2	Se	83.6	96.1	99.4
2-4-C2	Test-2	Test-4	C	Test-2	Sp	94.8	97.9	99.9
2-4-E1	Test-2	Test-4	E1	Test-2	Se	80.2	93.9	99.7
2-4-E1	Test-2	Test-4	E1	Test-2	Sp	94.3	97.1	99.5
2-4-E1	Test-2	Test-4	E1	Test-4	Se	64.3	83.1	95.1
2-4-E1	Test-2	Test-4	E1	Test-4	Sp	96.4	98.8	99.9
2-4-E2	Test-2	Test-4	E2	Test-2	Se	77.9	91.3	99.4
2-4-E2	Test-2	Test-4	E2	Test-2	Sp	96.2	98.4	99.9
2-4-E2	Test-2	Test-4	E2	Test-4	Se	64.68	86.5	99.26
2-4-E2	Test-2	Test-4	E2	Test-4	Sp	97.82	99.5	99.98
2-4-E3	Test-2	Test-4	E3	Test-2	Se	74.66	89.33	97.96
2-4-E3	Test-2	Test-4	E3	Test-2	Sp	93.07	97.09	99.55
2-4-E3	Test-2	Test-4	E3	Test-4	Se	59.12	77.63	93.72
2-4-E3	Test-2	Test-4	E3	Test-4	Sp	94.15	97.42	99.35
3-4-C	Test-3	Test-4	C	Test-3	Se	56.86	74.48	90.62
3-4-C	Test-3	Test-4	C	Test-3	Sp	93.72	97.01	98.87
3-4-C	Test-3	Test-4	C	Test-4	Se	67.9	77.48	85.45
3-4-C	Test-3	Test-4	C	Test-4	Sp	94.49	97.41	99.08
3-4-E1	Test-3	Test-4	E1	Test-3	Se	77.17	91.38	98.65
3-4-E1	Test-3	Test-4	E1	Test-3	Sp	96.41	98.32	99.45
3-4-E1	Test-3	Test-4	E1	Test-4	Se	72.48	86.99	96.03
3-4-E1	Test-3	Test-4	E1	Test-4	Sp	96.58	98.75	99.93
3-4-E2	Test-3	Test-4	E2	Test-3	Se	70.71	89.84	99.11
3-4-E2	Test-3	Test-4	E2	Test-3	Sp	96.92	99.15	99.95
3-4-E2	Test-3	Test-4	E2	Test-4	Se	80.62	95.13	99.79
3-4-E2	Test-3	Test-4	E2	Test-4	Sp	98.34	99.66	99.99
3-4-E3	Test-3	Test-4	E3	Test-3	Se	56.29	82.33	98.45
3-4-E3	Test-3	Test-4	E3	Test-3	Sp	95.04	98.55	99.94
3-4-E3	Test-3	Test-4	E3	Test-4	Se	61.2	83.61	96.11
3-4-E3	Test-3	Test-4	E3	Test-4	Sp	95.01	98.03	99.51

Table 14. Estimated values for prevalence of *Giardia*.

Population	Model code	LPI (5th perc.)	Median	UPI (95th prec.)
Non-diarrheic	1-2-C	5.11	7.08	9.50
	1-2-E1	0.70	3.18	7.12
	1-2-E2	2.68	5.36	9.16
	1-2-E3	8.40	12.01	16.30
	1-3-C	5.04	6.98	9.40
	1-3-E1	0.19	2.11	5.51
	1-3-E2	2.82	5.49	9.16
	1-3-E3	8.08	11.45	15.49
	1-4-C	4.97	6.88	9.22
	1-4-E1	0.52	2.92	6.76
	1-4-E2	2.52	5.10	8.77
	1-4-E3	7.86	11.24	15.28
	2-3-C	5.21	7.26	9.78
	2-3-E1	0.13	1.80	5.41
	2-3-E2	3.35	6.34	10.27
	2-3-E3	8.58	12.15	16.38
	2-4-C	4.80	6.72	9.09
	2-4-E1	0.31	2.50	6.72
	2-4-E2	2.41	5.22	9.35
	2-4-E3	8.10	11.62	15.86
	3-4-C	4.76	6.68	8.97
	3-4-E1	0.12	1.83	5.17
	3-4-E2	2.19	4.42	7.72
	3-4-E3	7.64	11.10	15.32
Diarrheic	1-2-C	8.51	13.33	19.21
	1-2-E1	7.53	13.63	21.63
	1-2-E2	8.60	14.15	20.96
	1-2-E3	9.67	16.31	24.60
	1-3-C	8.60	13.51	19.59
	1-3-E1	5.63	11.68	19.83
	1-3-E2	9.25	15.00	22.29
	1-3-E3	10.57	17.62	26.11
	1-4-C	8.64	13.46	19.55
	1-4-E1	8.07	15.13	23.88
	1-4-E2	9.45	15.35	22.69
	1-4-E3	9.73	16.61	24.97
	2-3-C	8.09	12.80	18.78
	2-3-E1	5.70	11.80	20.05
	2-3-E2	8.54	14.03	21.00
	2-3-E3	9.34	16.51	25.11
	2-4-C	8.16	12.85	18.75
	2-4-E1	6.82	13.29	21.80

Population	Model code	LPI (5th perc.)	Median	UPI (95th prec.)
	2-4-E2	8.72	14.37	21.49
	2-4-E3	9.09	15.68	23.90
	3-4-C	8.40	13.20	19.35
	3-4-E1	7.03	13.46	21.59
	3-4-E2	8.67	14.06	20.92
	3-4-E3	9.56	16.55	25.95

Table 15. Area under the autocorrelation plot, models 1-2-(C,E1-3)with *Cryptosporidium* detection results.

	Consensus	Expert1	Expert2	Expert3	Total
Test-2	12.19	24.58	12.23	23.69	72.68
sensitivity	4.70	17.03	5.56	11.20	38.49
specificity	7.49	7.54	6.67	12.491	34.18
Test-2	9.57	30.27	16.36	14.15	70.34
sensitivity	4.85	16.36	6.41	8.07	35.68
specificity	4.72	13.91	9.95	6.08	34.66
Prevalence	9.91	24.38	8.03	8.14	50.46
Pr1	5.22	10.91	3.63	3.89	23.65
Pr2	4.70	13.47	4.40	4.25	26.81
Total	31.66	79.23	36.62	45.97	193.48

Table 16. Area under the autocorrelation plot for sensitivity (*Giardia* detection results and consensus prior-distribution)

MODEL	TEST-3	TEST-2	TEST-4	TEST-1	Total
1-4-C	-	-	3.93	4.40	8.33
1-3-C	3.65	-	-	4.78	8.43
2-3-C	3.62	4.84	-	-	8.46
2-4-C	-	4.84	3.83	-	8.67
1-2-C	-	4.64	-	8.59	13.23
3-4-C	6.79	-	7.70	-	14.49
3-4-1-C	4.54	-	4.73	6.22	15.49
1-2-4-C	-	6.47	5.49	6.50	18.45
Total	18.60	20.79	25.68	30.49	95.56

Table 17. Area under the autocorrelation plot for specificity (*Giardia* detection results and consensus prior)

MODEL	TEST-2	TEST-3	TEST-1	TEST-4	Total
2- 4-C	-	-	5.42	5.61	11.04
1-4-C	5.71	-	-	6.20	11.91
2-3-C	7.32	5.72	-	-	13.04
1-3-C	-	8.65	13.69	-	22.34
1-2-C	8.67	-	18.45	-	27.13
3-4-C	-	41.10	-	55.02	96.12
1 -2 -4-C	106.34	-	122.17	130.30	358.81
3 -4 -1-C	-	190.71	197.14	219.22	607.07
Total	128.04	246.17	356.88	416.36	1147.45

Table 18. Area under the autocorrelation plot for *Giardia* prevalence (consensus prior)

MODEL	Pr1	Pr2	Total
2-3-C	3.32	2.82	6.13
2-4-C	3.54	3.26	6.80
1-4-C	3.38	3.70	7.07
1-2-C	3.38	4.01	7.39
3-4-1-C	3.29	4.10	7.39
1-3-C	3.96	3.45	7.41
1-2-4-C	3.97	5.10	9.06
3-4-C	3.65	5.80	9.44
Total	28.48	32.23	60.70

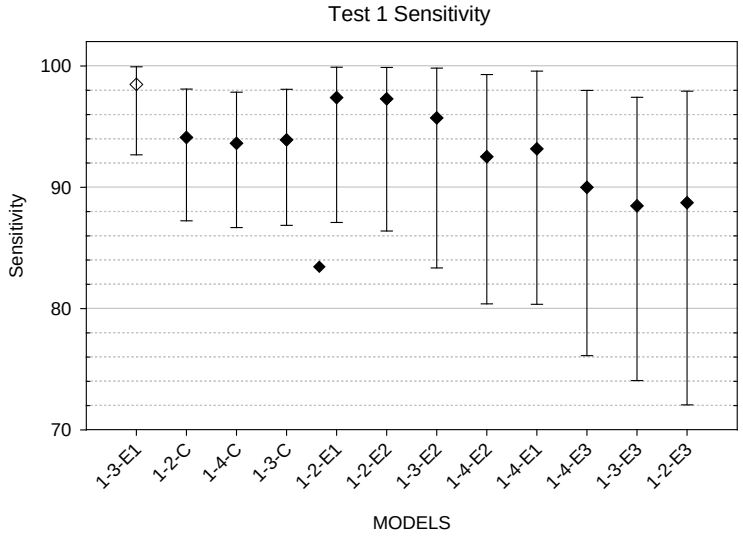


Figure 8. Posterior inferences of Test-1 sensitivity; median (±95% PI)

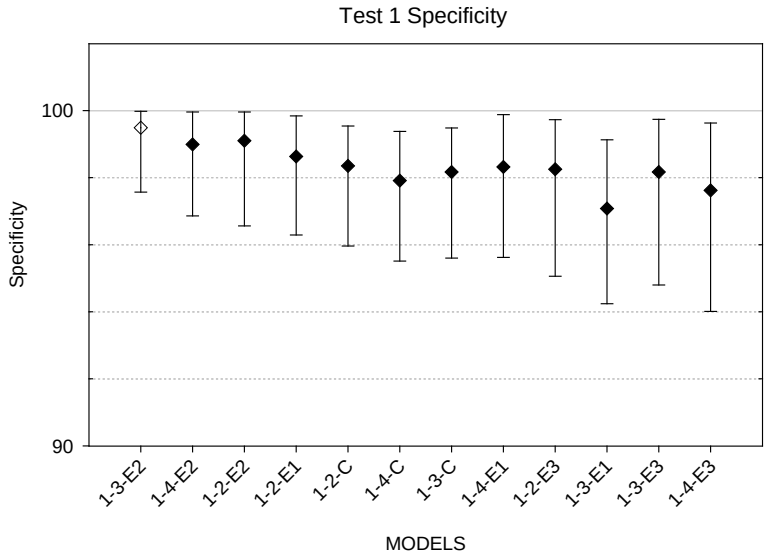


Figure 9. Posterior inferences of Test-1 specificity; median (±95% PI)

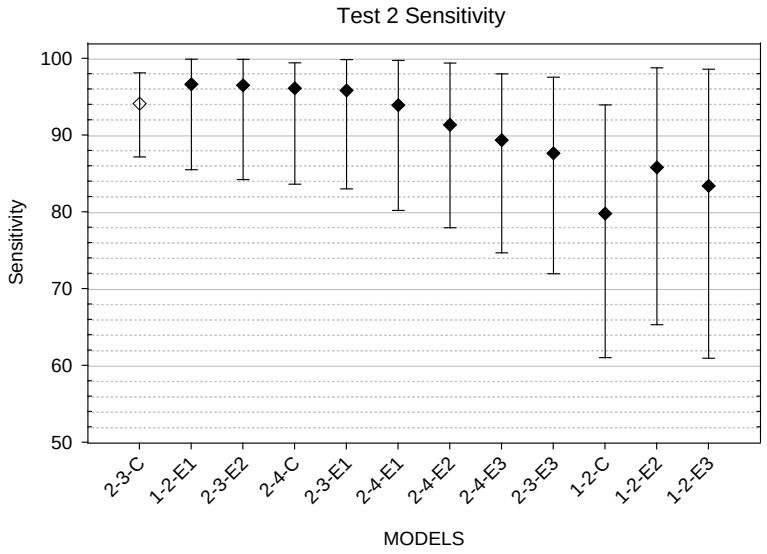


Figure 10. Posterior inferences of Test-2 sensitivity; median (±95% PI)

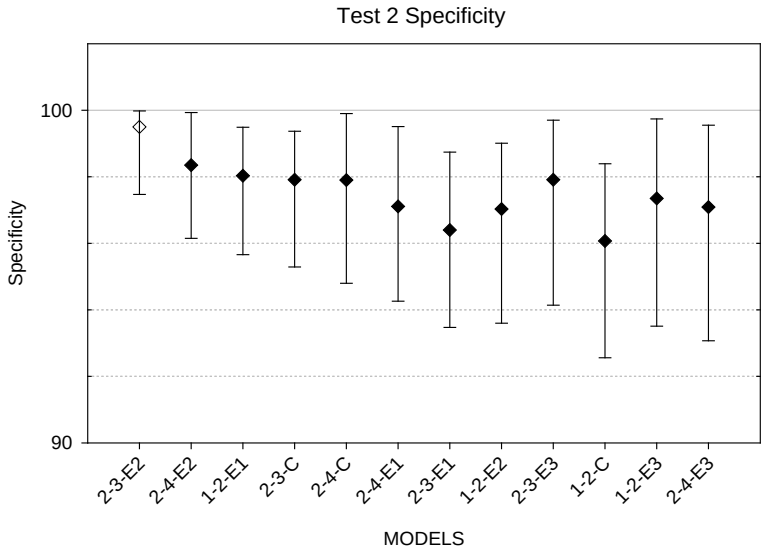


Figure 11. Posterior inferences of Test-2 specificity; median (±95% PI)

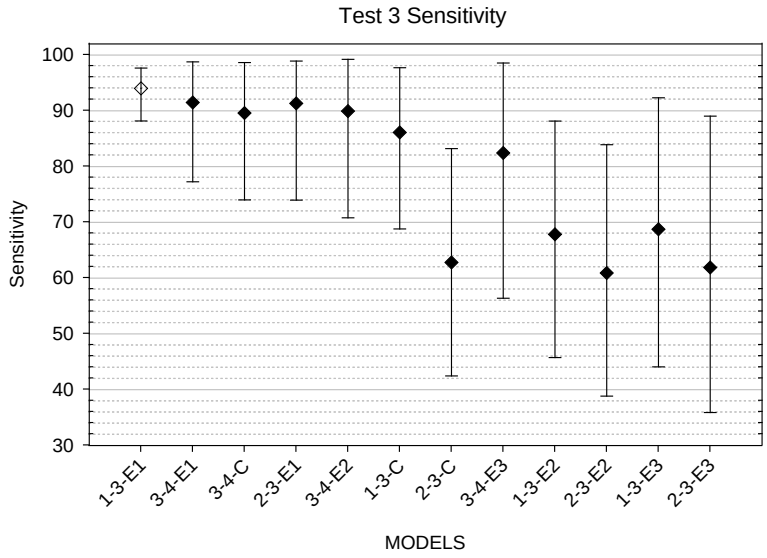


Figure 12. Posterior inferences of Test-3 sensitivity; median (±95% PI)

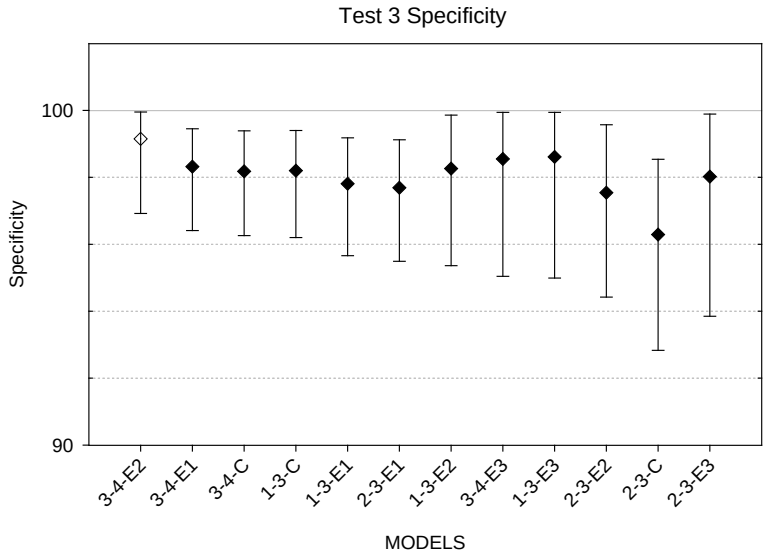


Figure 13. Posterior inferences of Test-3 specificity; median (±95% PI)

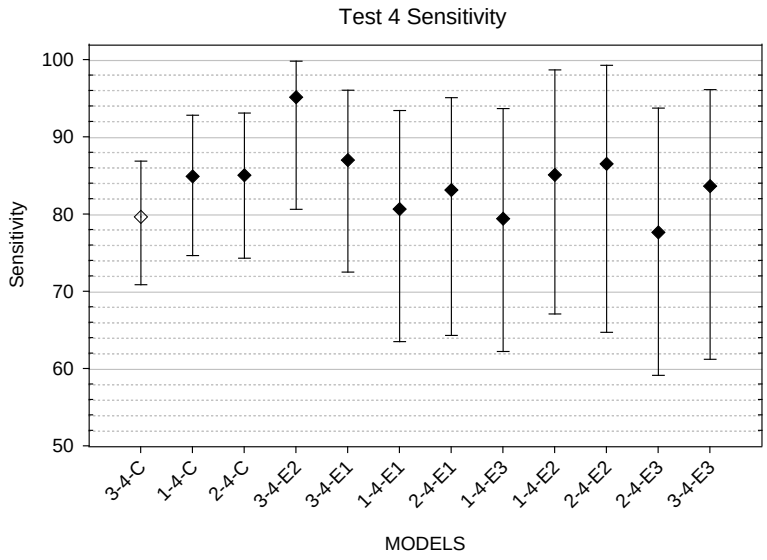


Figure 14. Posterior inferences of Test-4 sensitivity ; median (±95% PI).

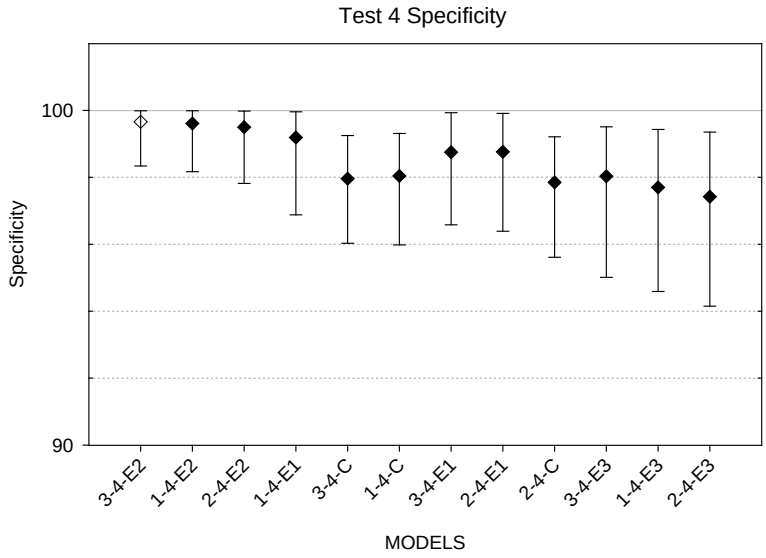


Figure 15. Posterior inferences of Test-4 specificity; median (±95% PI).

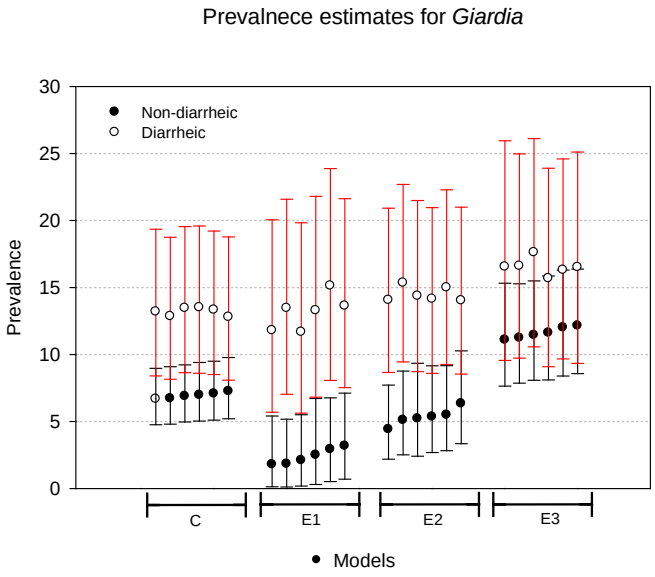


Figure 16. Estimates of *Giardia* Prevalence in the non-diarrheic vs. diarrheic populations; median (±95% PI; black = PI for non-diarrheic, red = PI for diarrheic).

4 DISCUSSION

4.1 LABORATORY TESTS RESULTS

When the four tests are used for the detection of *Giardia* , they detect relatively the same number of positive samples. According to this, we can hypothesize that performances of those tests are similar when the aim is detecting *Giardia.* In contrast, when we use Test-1 and Test-2 for detection of *Cryptosporidium* oocysts, the numbers differ; Test-2 detects more samples that are negative whileTest-1 detects more samples that are positive. Since, at this point, since sensitivity and specificity values are not yet available, it is no possible to know which of them are truly positive or negative, at this point is not possible to conclude which test performed better.

4.2 PRIOR DISTRIBUTIONS

Using consistency of the answers as indicator of question quality, we concluded that all experts understood the questions and provided answers consistent with their experience and background. Regarding the elicitation of prior distribution of sensitivity, this parameter had the most variations among experts. Expert 1 and 2 agreed in the sensitivity of Test-1 when used for detection of *Giardia;* they gave it a high sensitivity value with narrow lower confidence limits. For instance Johnson et al (2003), as well as other studies, reported that Test-1 detected the largest number of cases of *Giardia* and *Cryptosporidium,* thus the authors decided to use Test-1 as the reference test for calculating sensitivity and specificity of other lateral flow based and micro-plate tests (Zimmerman & Needham, 1995; Aziz, Beck, Lux, & Hudson, 2001). Expert 1 believed that Test-3 and Test-4 are not as sensitive as Test-1 and Test-2 tests, this is consistent with our hypothesis that opinions are related, at least in part, with the use of Test-1 as a reference test in other studies. As expected, all experts gave high values of specificity and narrow lower confidence

limits for all tests. These congruent values increase the reliability of the questions. As discussed before, one of our assumptions was that all tests have the same biological principle of identification, which makes the specificities correlated. According to these, we expected to obtain similar values in the elicited values of specificity.

There are also variations in the elicited values of prevalence. The three experts have different ways to see the distribution of the prevalence. For expert 1, all prevalence should be 0, but the expert's confidence upper value is higher when the texture of the sample is abnormal. In the other hand, for expert 2 the prevalence of *Giardia* is higher than the prevalence of *Cryptosporidium.* In addition, this expert also believes that is more probable to detect the parasites in abnormal samples. Finally, expert 3 believes that prevalence of *Giardia* is higher than prevalence of *Cryptosporidium,* and prevalence do not change across populations. This elicited values reflect at least in part what is found in the literature with prevalence variations according to the used tests or to the origin of the samples.

We expected to have variations among expert opinions, since our source of information are more than one expert. The experts may have their own sources of information that always influenced the perception of the reality about the parameters. The final elicited values are the results of an intricate reasoning; where previous readings, experience and background are mixed in different proportions, within the expert's minds. We may expect that for a particular disease where lots of research has been made, the experts' opinions would trend to convergence when asked about a parameter of the disease. In the same manner, the variation in opinion would decrease relatively to the amount accessible information.

4.3 POSTERIOR DISTRIBUTIONS INFERENCES

The criteria we used to choose the best estimator of the parameters are based in the three main characteristics of the performance of the models: identifiability, convergence, and autocorrelation. Then the best models for estimation of parameter are those with more degrees of freedom, rapid and stable convergence and with low autocorrelation. According to this, those are models with less covariance terms, with more informative priors, and lower autocorrelation values. Because there is no evidence to support the qualification or disqualification of any of the experts' prior distributions, the best models for estimation of the parameters are those extracted from the models using the consensus prior-distribution. This consensus prior distribution gave equal weight to all opinions and buffered the tails of the distributions, allowing the model to draw posterior distributions more precisely.

4.3.1 Test-1 and Test-2 for *Cryptosporidium* detection model

The best estimations of Test-1 and Test-2 parameters were extracted from the posterior distribution obtained by using the consensus prior distributions.

The sensitivity of Test-1 for detection of *Cryptosporidium* was 87.7% (78.6-94.3), which is much lower than the sensitivity reported in other studies (Johnston, Ballard, Beach, Causer, & Wilkins, 2003; Garcia & Shimizu, 1997; Zimmerman & Needham, 1995). The sensitivity of Test-2 was even lower than Test-1 (68.0% (37.2-98.7)). This was expected, since the number of positive samples detected by Test-2 was lower than those detected by Test-1. The possible causes of this low sensitivity can be related, at least in part, to a combination of issues with the preparation and reading of slides. Because of the lack of adherence of samples to the pretreated slide, loss of oocysts may occur, which would be particularly problematic in samples with few oocysts. However, the difference in the number of positive samples of Test-1 and Test-2 detecting *Giar-*

dia (21 and 22 respectively) may indicate that this is not the problem; one can expect that, as with the *Cryptosporidium* oocysts, also the *Giardia* cysts were washed away in the preparation. Other possible cause of the difference between sensitivities can be related to the reading process. The brighter background would make difficult the detection of the small oocysts, perhaps failing in detect true positive samples. Specificity values for Test-1 and Test-2 are 97.3% (94.7-99.5) and 99.1% (97.2-99.9) respectively, these values, as for sensitivity values, are lower than reported (Johnston, Ballard, Beach, Causer, & Wilkins, 2003). The specificity of these tests may be affected by the specificity of the antibody, by changes in the morphologic characteristics of the diagnostics forms, and by the skills of the technician. Any feature that affects the correct identification of the oocyts under the microscopic examination can affect the specificity of the tests. The prevalence of *Cryptosporidium* in the non-diarrheic population was significantly lower than in the diarrheic population (2.3% (1.4-4.1) vs. 4.8% (2.4-9.5)). These results are consistent with the fact that, even when the patient is infected with *Cryptosporidium*, this patient does not necessarily show abnormality of fecal texture (O'Hara & Chen, 2011; Ballweber, Panuska, Huston, Vasilopulos, Pharr, & Mackin, 2009).

4.3.2 Sensitivity and Specificity of Test-1 for detection of *Giardia*

All experts expressed to have experience with Test-1 and Test-4, giving informative priors. Thus the best model for estimation of Test-1 sensitivity and specificity is 1-4-C This model had only one extra covariance parameters in their specificity. In addition, this model presented the lower autocorrelation value when compared with other models containing Test-1 inputs.

The sensitivity of Test-1 for detection of *Giardia* was 93.6% (86.7-97.8), which is low when compared with the prior distribution and with other studies (Aziz, Beck, Lux, & Hudson, 2001;

Garcia & Shimizu, 1997; Johnston, Ballard, Beach, Causer, & Wilkins, 2003). These studies commonly used Test-1 as a reference test, giving it a default perfect sensitivity and specificity. The specificity of Test-1 for detection of *Giardia* is 97.91% (95.51-99.38). We expected to obtain a high specificity, not only because our experts agreed to high values of specificity, but also because the biological mechanism of the test uses monoclonal antibodies, which targets very specific proteins in the cyst-wall.

4.3.3 Sensitivity and Specificity of Test-2 for detection of *Giardia*

The better estimators for sensitivity and specificity of Test-2 test are extracted from the model 2-4-C2-4-C. This model has only one covariate terms and includes more informative prior distributions. In this study, we report the sensitivity and specificity of Test-2 test for the first time.

The sensitivity of Test-2 test was 96.1% (83.6-99.4). This value is practically equal to the sensitivity of Test-1 (93.6% (86.6-97.8)), what is explained by the fact that both tests uses the same biological principle and the same procedure for detection of antigens.

In the same manner, the specificity of Test-2 test (97.9% (94.8-99.9)) is practically the same that specificity of Test-1 (97.9% (95.5-99.4)). This enforces the conclusion that both tests are equally accurate identifying *Giardia* cysts in fecal samples of dogs and cats.

4.3.4 Sensitivity and Specificity of Test-3 for detection of *Giardia*

Taking in account the same parameters (identifiability, convergence, and autocorrelation) for choosing the best estimators of sensitivity and specificity, the best models was 1-3-C. informative priors.

The sensitivity of Test-3 test for detection of *Giardia* was 86.0% (68.7-97.6). The values obtained from models with non informative prior distributions were much lower. The uniform

distributions of non informative prior distributions dragged the posterior distribution towards lower values.

The specificity of Test-3 test was 98.2% (96.2-99.4). This value does not differ with the value of sensitivity obtained using other prior distributions. We obtained a higher number of negative results, which make the prior distributions affect in less proportion the posterior inferences.

The specificity obtained is comparable with results reported by Ungar et al (1984), where the sensitivity and specificity was 92% and 98% respectively. Even though the referenced study is old, this study is particularly interesting because the authors did not use DFA test are reference. Instead, for the positive results, they used samples that were confirmed by direct microscopic examination or intestinal biopsy, and for the negative results, they use samples from healthy patients that had no evidence of giardiasis.

We conclude that the specificity of Test-3 test is not different to the specificity of both DFA tests.

4.3.5 Sensitivity and Specificity of Test-4 for detection of *Giardia*

In the same way that the model Test-1-Test-4 with consensus prior was the best model for the estimation of sensitivity and specificity of MerifluorTest-1, this model was also the best model for the estimation of sensitivity and specificity of Test-4.

The sensitivity of Test-4 test was 84.8% (74.6-92.8). The sensitivity of Test-4 test is similar to the sensitivity of Test-3. This is similar to the reported by Mekaru R.S. et al (2007) and , where the sensitivity of snap test was 85.3%. The specificity of Test-4 test was 98.04% (96.08-99.32).This value is similar to the other tests specificities. The specificity of Test-4 test was 98.04% (96.08-99.32).This value is similar to the other tests specificities.

Both Test-3 and Test-4 performed similarly. This is not surprising since these test shares many features in their principles of action. The main difference with Test-1 and Test-2 is the way of declaring the true positives. While Test-1 and Test-2 uses the direct identification of cyst forms, Test-3 and Test-4 use a colorimetric scale. For samples with lower concentration of cysts, the colorimetric change may not detectable, thus decreasing the probability of finding a true positive sample.

4.3.6 Prevalence of *Giardia*

As shown in Table 14 it is notable that all models converge to similar results with some variations depending on the prior distribution used, even those models with lower performance. However, to be consistent with the criteria used for the estimation of other parameters, the best model for the estimation of prevalence was 1-4-C.

We identify that the prevalence of *Giardia* in the populations differs significantly. For the population with non-diarrheic samples, the prevalence *Giardia* was 6.9% (5.0-9.2), and for the population with diarrheic samples, the prevalence of *Giardia* was 13.5% (8.6-19.6).

Comparing this results to other studies with similar target populations. For instance, Carlin et al. (2006) found a prevalence of *Giardia* of 15.6% and 10.8% in symptomatic dogs and cats respectively. This result is similar to what we find in diarrheic (~symptomatic) samples. This study used IDEXX Snap test (TestusedTest-4) throughout veterinary clinics on the US. In other study where samples attending a Veterinary Hospital in Pisa, Italy (Bianciardi, Papini, Giuliani, & Cardini, 2004), the researchers found a higher prevalence of *Giardia*,17.52% and 37.5%, in both diarrheic and not-diarrheic groups , respectively. In Belgium in dogs with gastrointestinal problems, the prevalence of *Giardia* was 18.1% (Claerebout, et al., 2004).

4.4 FINAL COMMENTS

Diagnostic tests play major roles in the practice of medicine and research. Its applications include clinical diagnostic aid, surveillance activities, certification of freedom of disease, prevalence estimation, risk assessment, etc. (Greiner & Gardner, 2000). Given the importance of these tools, we may want to know how well they perform under particular conditions. Indeed, it is possible to evaluate the accuracy of diagnostic tests using Se and Sp.

The Bayesian latent class analysis was an effective tool for estimating the accuracy of the diagnostic test of interest in absence of a gold standard. Instead of coding and running complex models with all tests included at once, we used the simpler and more effective 2-tests 2-populations model for comparing pairs of tests. We effectively used indicators of model performance to choose the best models for estimation of inferences. The three main characteristics of the performance of the models were identifiability, convergence, and autocorrelation. Consequently, the best models for estimation of a parameter were those with more degrees of freedom—more informative priors and less covariance terms, more rapid and stable convergence, and low autocorrelation values. The use of a consensus prior distribution—constructed from informative prior distributions—was effective in combining different opinions and experiences about a parameter, even when there is lack of accessible information. The inclusion of a third test in the model fails to increase the identifiability of the models. In contrast, this models were slower in reaching convergence and present high autocorrelation. This was caused because the specificities of all tests were not conditional independent. This required the inclusion of more covariance terms limiting the degrees of freedom available.

With this study, we were able to estimate accuracy values for four commercial diagnostic kits. Merifluor (Test-1) seems to be the best test of the four evaluated. With Merifluor, it is possible to

detect both *Giardia* and *Cryptosporidium* that are often found causing gastrointestinal disease in dogs and cats. IVD-DFA (Test-2) was designed to detect both parasites as well, but its sensitivity for detection of *Cryptosporidium* was significantly lower. The main disadvantage of DFA tests (Test-1 & Test-2) is that they required the availability of a fluorescence microscopy , which limits their use in small practices or in the field. They also require more time for reading the slides and proper training for identification of cysts and oocysts (more than fecal flotation?). According to this, the Test-1 and Test-2 are tools that may be effective for diagnosis of *Giardia* and *Cryptosporidium* in major laboratories or in hospital settings.

Microwell ELISA test (Test-3) is a rapid and practical test. The time to get results is approximately 2 hours and it is possible to read the results without a spectrophotometer, using a visual scale (provided), which increases the range of its use. However, the sensitivity of this test was significantly lower than the DFA tests (Test-1 & Test-2). SNAP (Test-4) test is a more rapid test that is easy to use; results can be read in less than 10 minutes. Regarding sensitivity and specificity, this test is similar to Test-3. The main difference between those two may be the ease to use regarding the number of samples to process. Test-3 seems to be more convenient for reading batches of samples—such as for screening of populations of kernels and shelters—since its presentation in 96-well racks and the use of a single control positive and negative for each batch, make it preferable. On the other hand, Test-4 seems to be more suitable for initial screening of suspicious infected individuals—such as in patients attending medical consultation in small practices reporting gastrointestinal problems compatible with *Giardia* infection. Their lower sensitivity compared to the DFA tests (Test-1 and Test-2) and the fact that these tests only detect *Giardia* might be their major disadvantages.

5 BIBLIOGRAPHY

Adamska, M., Leońska-Duniec, A., Maciejewska, A., Sawczuk, M., & Skotarczak, B. (2010). Comparison of efficiency of various DNA extraction methods from cysts of Giardia intestinalis measured by PCR and TaqMan real time PCR. *Parasite* , 299-305.

Ankarklev, J., Jerlstrom-Hultqvist, J., Ringqvist, E., Troell, K., & Svard, S. G. (2010). Behind the smile: cell biology and disease mehanism of Giardia species. *Nature Reviews Microbiology* , 413-422.

Armson, A., Reynoldson, J., & Thompson, R. (2003). A Review of Chemotherapeutic Approaches to the treatment of Cryptosporidium. In A. Thompson, A. Armson, & U. M. Ryan, *Cryptosporidium* (pp. 395-403). Amsterdam: Elsevier.

Aziz, H., Beck, C., Lux, M., & Hudson, M. (2001). A comparison study of different methods used in the detection of Giardia lamblia. *Clinical Laboratory Science* , 150-154.

Babaei, Z., Oormazdi, H., Rezaie, S., Rezaeian, M., & Razmjou, E. (2011). Giardia intestinalis: DNA extraction approaches to improve PCR results. *Experimental Parasitology* , 159-162.

Bajer, A., Bednarska, M., & Rodo, A. (2011). Risk factors and control of intestinal parasite infections in sled dogs in Poland. *Veterinary Parasitology* , 343-350.

Ballweber, L. R., Panuska, C., Huston, C. L., Vasilopulos, R., Pharr, T., & Mackin, A. (2009). Prevalence of and risk factors associated with shedding of Cryptosporidium felis in domestic cats of Mississippi and Alabama. *Veterinary Parasitology* , 306-310.

Ballweber, L. R., Xiao, L., Bowman, D. D., Kahn, G., & Cama, V. A. (2010). Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends in Parasitology* , 180-189.

Barr, S., Bowman, D., Heller, R., & Erb, H. (1993). Efficacy of albendazole against giardasis in dogs. *American Journal of Veterinary Research* , 926-928.

Beiromvand, M., Akhlaghi, L., Massom, S. H., Meamar, A. R., Motevalian, A., Oormazdi, H., et al. (2013). Prevalence of zoonotic intestinal parasites in domestic and stray dogs in a rural area of Iran. *Preventive Veterinary Medicine* , 162-167.

Berghoff, N., & Steiner, J. M. (2011). Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. *Veterinary Clinics of Noth America: Smal Animal Practice* , 311-328.

Bianciardi, P., Papini, R., Giuliani, G., & Cardini, G. (2004). Prevalence of Giardia antigen in stool samples from dogs and cats. *Revue de Médecine Vétérinaire* , 417-421.

Branscum, Gardner, & Johnson. (2005). Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Preventive Veterinary Medicine* , 145-163.

Caccio, S. M., Beck, R., Almeida, A., Bajer, A., & Pozio, E. (2010). Identification of Giardia species and Giardia duodenalis assemblages by sequence analysis of the 5.8S rDNA gene and internal transcribed spacers. *Parasitology* , 919-925.

Carlin, E., Bowman, D., Scarlett, J., Garrett, J., & Lorentzen, L. (2006). Prevalence of Giardia in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP Giardia test. *Veterinary Therapeutics : Research in Applied Veterinary Medicine* , 199-206.

Causapé, A., Quílez, J., Sánchez-Acedo, C., & Cacho, E. d. (1996). Prevalence of intestinal parasites, including Cryptosporidium parvum, in dogs in Zaragoza city, Spain. *Veterinary Parasitology* , 161-167.

Cavalier-Smith, T. (2003). Protist phylogeny and the high-level classification of Protozoa. *European journal of protistology* , 338-348.

Christensen, J., & Gardner, I. A. (2000). Herd-level interpretation of test results for epidemiologic studies of animal diseases. *Preventive Veterinary Medicine* , 83-106.

Christensen, R., Johnson, W., Branscum, A., & Hanson, T. E. (2011). *Bayesian ideas and data analysis.* Boca Raton, FL: Tylor and Francis Group.

Claerebout, E., S. Casaert, Dalemans, A.-C., Wilde, N. D., Levecke, B., Vercruysse, J., et al. (2004). Giardia and other intestinal parasites in different dog populations in Northern Belgium. *Veterinary Parasitology* , 41-46.

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and Psychological Measurement* , 37-46.

Cotton, J. A., Beatty, J. K., & Buret, A. G. (2011). Host parasite interactions and pathophysiology in Giardia infections. *International Journal for Parasitology , 41* (9), Pages 925-933.

Deng, M. J., Ji, X. C., Xiao, X. Z., Sun, T., Wu, Z. X., Zheng, X. L., et al. (2014). Immuno-PCR for Detection of Giardia lamblia Cysts in Water. *Journal of AOAC International* , 561-566.

Deng, M.-Q., & Cliver, D. O. (1999). Rapid DNA extraction methods and new primers for randomly amplified polymorphic DNA analysis of Giardia duodenalis. *Journal of Microbiological Methods* , 193-200.

Dubná, S., Langrová, I., Nápravník, J., Jankovská, I., Vadlejch, J., Pekár, S., et al. (2007). The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. *Veterinary Parasitology* , 120-128.

Ederli, B. B., Ederli, N. B., Oliveira, F. C., Quirino, C. R., & Carvalho, C. B. (2008). Fatores de risco associados à infecção por Cryptosporidium spp. em cães domiciliados na cidade de Campos

dos Goytacazes, Estado do Rio de Janeiro, Brasil. *Revista Brasileira de Parasitologia Veterinária* , 250-266.

el-Ahraf, A., Tacal, J. J., Sobih, M., Amin, M., Lawrence, W., & Wilcke, B. (1991). Prevalence of cryptosporidiosis in dogs and human beings in San Bernardino County, California. *Journal of the American Veterinary Medical Association* , 631-634.

Elitok, B., Elitok, Ö. M., & Pulat, H. (2008). Efficacy of Azithromycin Dihydrate in Treatment of Cryptosporidiosis in Naturally Infected Dairy Calves. *Journal of Veterinary Internal Medicine* , 590-593.

Enoe, C., Geordais, M. P., & Johnson, W. O. (2000). Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Preventive Veterinary Medicine* , 61-81.

Enøe, C., Geordais, M. P., & Johnson, W. O. (2000). Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. *Preventive Veterinary Medicine* , 61-81.

Fayer, R. (2010). Taxonomy and species delimitation of Cryptosporidium. *Experimental Parasitology* , 90-97.

Feiss, J. L., Levin, B., & Paik, M. C. (2003). *Statisticals methods for rates and proportions* (Third edition ed.). New Jersey: Jhon Wiley & Sons.

Feng, Y., & Xiao, L. (2012). Giardia. In D. Liu, *Molecular Detection of Human Parasitic Pathogens* (pp. 77-90).

Feng, Y., & Xiao, L. (2011). Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. *Clinical Microbiology Reviews* , 110-140.

Garcia, L. S., & Shimizu, R. Y. (1997). Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of Giardia lamblia and Cryptosporidium parvum in human fecal specimens. *Journal of Clinical Microbiology* , 1526-1529.

Gardner, Stryhn, Lind, & Collins. (2000). Conditional dependence between tests affects the diagnosis and surveilance of animal disease. *Preventive Veterinaty Medicine* , 107-122.

Gardner, T., & Hill, D. R. (2001). Treatment of Giardiasis. *Clnical Microbiology Reviews* , 114-128.

Gargala, G. (2008). Drug treatment and novel drug target against Cryptosporidium. *Parasite* , 275-281.

Georgiadis, M. P., Johnson, W. O., & Gardner, I. A. (2005). Sample size determination for estimation of the accuracy of two conditionally independent tests in the absence of a gold standard. *Preventive Veterinary Medicine* , 1-10.

Giangaspero, A., Iorio, R., Paoletti, B., Traversa, D., & Capelli, G. (2006). Molecular evidence for Cryptosporidium infection in dogs in Central Italy. *Parasitology Research* , 297-299.

Goka, A., Rolston, D., Mathan, V., & Farthing, M. (1990). The relative merits of faecal and duodenal juice microscopy in the diagnosis of giardiasis. *Transactions of the Roya Society of Tropical Medicine and Hygiene* , 66-67.

Gokbulut, C., Bilgili, A., Hanedan, B., & McKellar, Q. (2007). Comparative plasma disposition of fenbendazole, oxfendazole and albendazole in dogs. *Veterinary Parasitology* , 279-287.

Greiner, M., & Gardner, I. (2000). Application of diagnostic tests in veterinary epidemiologic studies. *Preventive veterinary medicine* , 43-59.

Greiner, M., & Gardner, I. (2000). Epidemiologic issues in the validation of a veterinary diagnostic tests. *Preventive Veterinary Medicne* , 3-22.

Greiner, M., Pfeiffer, D., & Smith, R. (2000). Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine* , 23-41.

Guy, R. A., Payment, P., Krull, U. J., & Horgen, P. A. (2003). Real-Time PCR for Quantification of Giardia and Cryptosporidium in Environmental Water Samples and Sewage. *Applied and Environmental Microbiology* , 5179-5185.

Hackett, T., & Lappin, M. R. (2003). Prevalence of Enteric Pathogens in Dogs of North-Central Colorado. *Jorunal of the American Animal Hospitals Asociations* , 52-56.

Hui, S. L., & Walter, S. D. (1980). Estimating error rates of diagnostic tests. *Biometrics* , 167-171.

Integrated Taxonomic Information System. (2013, March 8). *Integrated Taxonomic Information System*. Retrieved April 8, 2013, from http://www.itis.gov/

Jacobson, R., & Wright, P. (2013). Principles and methods of validation of diagnostic assays for infectious diseases. In W. O. Health, *Manual of diagnostic tests and vaccines for terrestrial animals.* OIE.

Jian, F., Qi, M., X. H., Wang, R., Zhang, S., Dong, H., et al. (2014). Occurrence and molecular characterization of Cryptosporidium in dogs in Henan Province, China. *BMC Veterinary Research* .

Johnson, W. O., Gardner, I. A., Metoyer, C. N., & Branscum, A. J. (2009). On the interpretation of test sensitivity in the two-test two-population problem: Assumptions matter. *Preventive Veterinary Medicine* , 116-121.

Johnson, W. O., Gastwirth, J. L., & Pearson, L. M. (2001). Screening without a “Gold Standard”: The Hui-Walter Paradigm Revisited. *American Journal of Epidemiology* , 921-924.

Johnston, S. P., Ballard, M. M., Beach, M. J., Causer, L., & Wilkins, P. P. (2003). Evaluation of Three Commercial Assays for Detection of Giardia and Cryptosporidium Organisms in Fecal Specimens. *Journal of Clinica Microbiology* , 623-226.

José L. Alonso, I. A. (2011). Development and evaluation of a real-time PCR assay for quantification of Giardia and Cryptosporidium in sewage samples. *Applied Microbiology and Biotechnology* , 1203-1211.

Katagiri, S., & Oliveira-Sequeira, T. C. (2008). Prevalence of Dog Intestinal Parasites and Risk Perception of Zoonotic Infection by Dog Owners in São Paulo State, Brazil. *Zoonoses and Public Health* , 406-413.

Kirkpatrick, C., & Farrell, J. (1984). Feline giardiasis: observations on natural and induced infections. *American Journal of Veterinary Research* , 2182-8.

Kirkpatrik, C. E. (1987). Giardiasis. *The Veterinary Clinics of North America: Small Animal Practice* , 1377-1387.

Koehler, A. V., Jex, A. R., Haydon, S. R., Stevens, M. A., & Gasser, R. B. (2013). Giardia/giardiasis — A perspective on diagnostic and analytical tools. *Biotecnology Advances* .

Lappin, M. R. (2004). Enteric protozoal diseases. *Veterinary Clinics of North America: Small Animal Practice* , 81-88.

Lau, A., Lam, N., Piscitelli, S., & L. Wilkes, L. D. (1992). Clinical pharmacokinetics of metronidazole and other nitro-imidazole anti-infectives. *Clinical pharmacokinetics* , 328-364.

Lilford, R. J., Pauker, S. G., Braunholtz, D. A., & Chard, J. (1998). Decision analysis and the implementation of research findings. *British medical journal* , 405-409.

Lora R. Ballweber, C. P. (2009). Prevalence of and risk factors associated with shedding of Cryptosporidium felis in domestic cats of Mississippi and Alabama. *Veterinary Parasitology* , 306-310.

Lucio-Forster, A., Griffiths, J. K., Cama, V. A., Xiao, L., & Bowman, D. D. (2010). Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends in Parasitology* , 174-179.

Luisa Rambozzi, A. M. (2007). Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. *Journal of Feline Medicine & Surgery* , 392-396.

MacPherson, J., Eckstein, P., Scoles, G., & Gajadhar, A. (1993). Variability of the random amplified polymorphic DNA assay among thermal cyclers, and effects of primer and DNA concentration. *Molecular and Cellular Probes* , 293-299.

Mark-Carew, M. P., Adesiyun, A. A., Basu, A., Georges, K. A., Pierre, T., Tilitz, S., et al. (2013). Characterization of Giardia duodenalis infections in dogs in Trinidad and Tobago,. *Veterinary Parasitology* , 199-202.

Marks, S. L., Hanson, T. E., & Melli, A. C. (2004). Comparison of direct immunofluorescence, modified acid-fast staining, and enzyme immunoassay techniques for detection of Cryptosporidium spp in naturally exposed kittens. *Journal of the American Veterinary Medical Asociation* , 1549-1553.

Mekaru, S. R., Marks, S. L., Felley, A. J., Chouicha, N., & Kass, P. H. (2007). Comparison of Direct Immunofluorescence, Immunoassays, and Fecal Flotation for Detection of Cryptosporidium spp. and Giardia spp. in Naturally Exposed Cats in 4 Northern California Animal Shelters. *Journal of Veterinary Internal Medicine* , 959-965.

Miller, M., Howes, H., Kasubick, R., & English, A. (1970). Alkylation of 2-methyl-5-nitroimidazole. Some potent antiprotozoal agents. *Journal of Medicinal Chemistry* , 849.

Mohamed, A. S., Glickman, L. T., Jr., J. W., Lund, E., & Moore, G. E. (2013). Prevalence and risk factors for Giardia spp. infection in a large national sample of pet dogs visiting veterinary hospitals in the United States (2003–2009). *Veterinary Parasitology* , 35-41.

Morgan, U., J.A., R., & R.C.A., T. (1993). Activities of Several Benzimidazoles and Tubulin Inhibitors against Giardia spp. In Vitro. *Andetimycrobial Agents and Chemotherapy* , 328-331.

Mundim, M., Rosa, L., Hortêncio, S., Faria, E., Rodrigues, R., & Cury, M. (2007). Prevalence of Giardia duodenalis and Cryptosporidium spp. in dogs from different living conditions in Uberlândia, Brazil. *Veterinary Parasitology* , 356-359.

Navarrete-Vázquez, G., Cedillo, R., Hernández-Campos, A., Yépez, L., Hernández-Luis, F., Valdez, J., et al. (2001). Synthesis and antiparasitic activity of 2-(Trifluoromethyl)benzimidazole derivatives. *Bioorganic & Medicinal Chemistry Letters* , 187-190.

Niichiro Abe, Y. S. (2002). Cryptosporidium infection in dogs in Osaka, Japan. *Veterinary Parasitology* , 185-193.

O'Hara, S. P., & Chen, X.-M. (2011). The cell biology of Cryptosporidium infection. *Microbes and Infection* , 721-730.

O'Donoghue, P. J. (1995). Cryptosporidium and Cryptosporidiosis in man and animals. *International Journal for Parasitology* , 139-195.

Olson, M. E., & Heine, J. (2009). Synergistic Effect of Febantel and Pyrantel Embonate in Elimination of Giardia in a Gerbil Model. *Parasitology Research* , 135-140.

Omoruyi, B. E., Nwodo, U. U., Udem, C. S., & Okonkwo, F. O. (2014). Comparative Diagnostic Techniques for Cryptosporidium Infection. *molecules* , 2674-2683.

Pavlasek, I., & Ryan, U. (2007). The first finding of a natural infection of Cryptosporidium muris in a cat. *Veterinary Parasitology* , 349-352.

Payne, P. A., & Artzer, M. (2009). The biology and control of Giardia spp and Tritrichomonas foetus. *Veterinary Clinics of North America: Small Animal Practice* , 993-1007.

Pelayo, L., Fraga, J., Núñez, F. A., Mendoza, D., Torres, D. R., & Finlay, C. M. (2003). Genetic characterization by random amplified polymorphic DNA analysis (RAPD) of 18 isolates of Giardia lamblia obtained from day care children. *Experimental Parasitology* , 162-166.

Pluzer, J., Ongerth, J., & Karanis, P. (2010). Giardia taxonomy, phylogeny and epidemiology: Facts and open questions. *International Journal of Hygiene and Enviromental Health* , 321-333.

Ramirez, N. E., Ward, L. A., & Sreevatsan, S. (2004). A review of the biology and epidemiology of cryptosporidiosis in humans and animals. *Microbes and Infection* , 773-785.

Rishniw, M., Liotta, J., Bellosa, M., Bowman, D., & Simpson, K. (2010). Comparison of 4 Giardia Diagnostic Tests in Diagnosis of Naturally Acquired Canine Chronic Subclinical Giardiasis. *Journal of Veterinary Internal Medicine* , 293-297.

Rossignol, J.-F. (2010). Cryptosporidium and Giardia: Treatment options and prospects for new drugs. *Experimental Parasitology* , 45-53.

S. Barr, D. B. (1994). Efficacy of fenbendazole against giardasis in dogs. *American Journal of Veterinary Research* , 988-990.

Scorza, A. V., & Lappin, M. R. (2004). Metronidazole for the treatment of feline giardiasis. *Journal of Feline Medicine and Surgery* , 157-160.

Scorza, A. V., Brewer, M. M., & Lappin, M. R. (2003). Polymerase Chain Reaction for the Detection of Cryptosporidium Spp. in Cat Feces. *Journal of Parasitology* , 423-426.

Scorza, V., & Lappin, M. R. (2012). Cryptosporidiosis. In C. E. Greene, *Infectious diseases of the dog and the cat* (pp. 840-849). St. Louis, MO.: Elsevier.

Scorza, V., & Lappin, M. R. (2012). Giardiasis. In C. E. Greene, *Infectious Diseases of the Dog and Cat* (pp. 785-792). St. Louis, MO.: Elsevier/Saunders.

Scorza, V., & Tangtrongsup, S. (2010). Update on the diagnosis and management of Cryptosporidium spp nfections in dogs and cats. *Topics in Companion Animal Medicine* , 163-169.

Scorza, V., Brewer, M., & Lappin, M. (2003). Polymerase chain reaction for the detection of Cryptosporidium spp. in cat feces. *The Journal of Parasitology* , 423-426.

Smith, H. V., Nichols, R. A., & Grimason, A. M. (2005). ryptosporidium excystation and invasion: getting to the guts of the matter. *Trends in Parasitology* , 133-142.

Smith, R., & Slenning, B. (2000). Decision analysis: dealing with uncertainty in diagnostic testing. *Preventive Veterinary Medicine* , 139-162.

Sox, H. C. (1996). Probability theory in the use of diagnostic tests. *Annals of Internal Medicine* , 60-66.

Strand, E. A., Robertson, L. J., Hanevik, K., Alvsva, J. O., & Langeland, N. (2008). Sensitivity of a Giardia antigen test in persistent giardiasis following an extensive outbreak. *Clinical Microbiology and Infection* , 1069-1071.

Szklo, M., & Nieto, F. J. (2007). *Epidemiology: beyond the basics.* Sudbury: Jones and Barlett Publishers.

Tangtrongsup, S., & Scorza, V. (2010). Update on the diagnosis and management of Giardia spp infections in dogs and cats. *Topics in Companion Animal Medicine* , 155-162.

TDR Diagnostics Evaluation Expert Pannel. (2010). Evaluation of diagnostic tests for ingectious diseases: general principles. *Nature Reviews* , S17-S29.

Thompson, R., & P.T. Monis. (2004). Variation in Giardia: Implications for Taxonomy and Epidemiology. *Advances in Parasitology* , 69-137.

Thomson, R. C., Palmer, C. S., & O'Handley, R. (2008). The public health and clinical significance of Giardia and Cryptosporidium in domestic animals. *The Veterinary Journal* , 18-25.

Thomson, R. (2004). The zoonotic significance and molecular epidemiology of Giardia and giardiasis. *Veterinary Parasitology* , 15-35.

Toft, N., Jørgensen, E., & Højsgaard, S. (2005). Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Preventive Veterinary Medicine* , 19-33.

Trustfield, M. (2005). Diagnostic testing. In M. Trustfield, *Vetrinary epidemiology* (pp. 302-330). Ames: Blackwell.

Ungar, B. L., Yolken, R. H., Nash, T. E., & Quinn, T. C. (1984). Enzyme-Linked Immunosorbent Assay for the Detection of Giardia lamblia in Fecal Specimens. *The journal of infectious disease* , 90-97.

Uppal, B., Singh, O., Chadha, S., & Jha, A. K. (2014). A Comparison of Nested PCR Assay with Conventional Techniques for Diagnosis of Intestinal Cryptosporidiosis in AIDS Cases from Northern India. *Journal of Parasitology Research* .

Weber, R., Bryan, R. T., Bishop, H. S., Wahlquist, S. P., Sullivan, J. J., & Juranek, D. D. (1991). Threshold of detection of Cryptosporidium oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *Journal of Clinical Mycrobiology* , 1323-1327.

Westermarck, E., Skrzypczak, T., Harmoinen, J., Steiner, J. M., Ruaux, C. G., Williams, D. A., et al. (2005). Tylosin-Responsive Chronic Diarrhea in Dogs. *Journal of Veterinary Internal Medicine* , 177-186.

Wetzel, D. M., Schmidt, J., Kuhlenschmidt, M. S., Dubey, J. P., & Sibley, a. L. (2005). Gliding Motility Leads to Active Cellular Invasion by Cryptosporidium parvum Sporozoites. *Infection and Immunology* , 5379-5387.

Yang, D., Zhang, Q., Zhang, L., Dong, H., Jing, Z., Li, Z., et al. (2014). Prevalence and risk factors of Giardia doudenalis in dogs from China. *International Journal of Environmental Health Research* .

Yoder, J. S., & Beach, M. J. (2010). Cryptosporidium surveillance and risk factors in the United States. *Experimental Parasitology* , 31-39.

Zimmerman, S. K., & Needham, C. A. (1995). Comparison of conventional stool concentration and preserved-smear methods with Merifluor Cryptosporidium/Giardia Direct Immunofluorescence Assay and ProSpecT Giardia EZ Microplate Assay for detection of Giardia lamblia. *Journal of Clinical Microbiology* , 1942-1943.

Appendix I

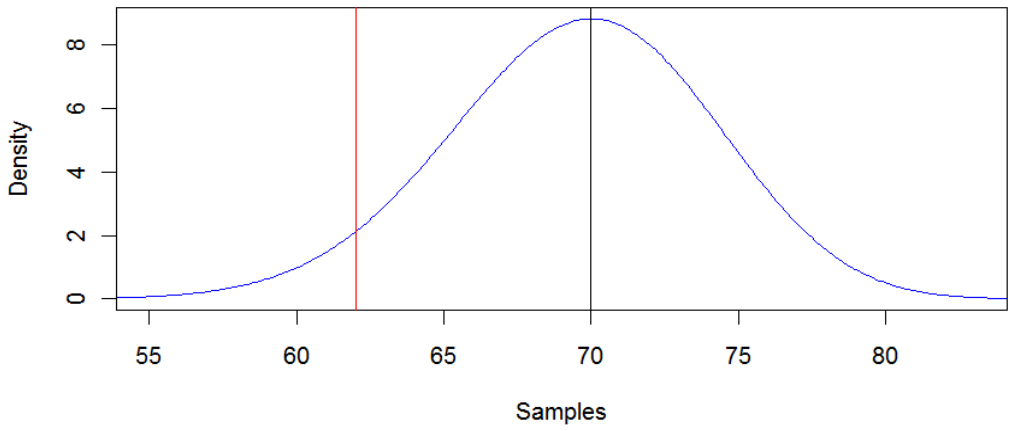
SURVEY FOR PRIOR DISTRIBUTIONS AND ELICITATION

TEST FOR EVALUATION

- Merifluor *Cryptosporidium*/*Giardia* IFA kit; Meridian Bioscience Inc., Cincinnati OH. (MERIFLUOR)
- Safepath (IVD) *Cryptosporidium*/*Giardia* Fecal DFA detection kit; IVD Research Inc., Carlsbad, CA. (SAFEPATH)
- Safepath (IVD) *Giardia* Antigen Detection Microwell ELISA; IVD Research Inc., Carlsbad, CA. (ELISA)
- Snap *Giardia* ; IDEXX Laboratories Inc., Westbrook, ME. (SNAP)

The following example illustrates how the answers will be used to simulate the probability distribution of repeating the tests thousands of times, this apply for the other examples as well.

Example 1 INFECTED SAMPLES CASE



According to an expert opinion and/or experience, if the test X is applied to **100 known INFECTED** samples, the most possible number of samples the test will detect as **positive** is 70 (mode, black line), and the expert is 95% sure the minimum number of samples the test would detect as **positive** is 62 (red line, percentile 5th)

For the following INFECTED SAMPLES cases:

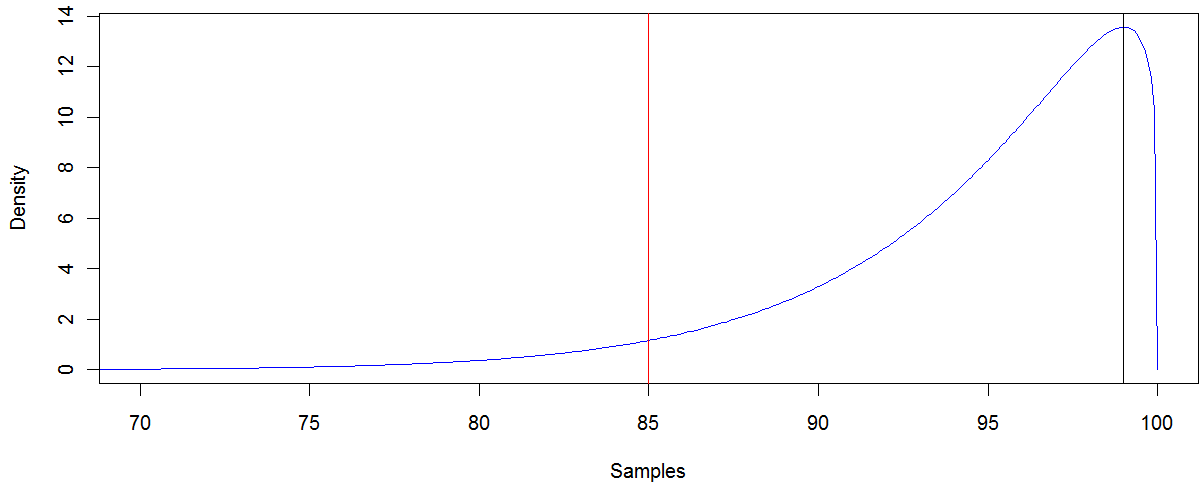
1. In the hypothetical case of applying, the test to 100 known **INFECTED** *Giardia* fecal samples from dogs and cats with the aim for the detection of *Giardia* **.**
    a. Using the above hypothetical case, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **positive?**
    b. In addition, according to your opinion and/or experience, what is the **MINIMUM** number out of these 100 samples that you are 95% sure will be **positive?**

TEST	MINIMUM NUMBER OF POSITIVES	MOST POSSIBLE NUMBER OF POSITIVES
MERIFLUOR		
SAFEPATH		
ELISA		
SNAP		

2. In the hypothetical case of applying, the test to 100 known **INFECTED** *Cryptosporidium* fecal samples from dogs and cats with the aim for the detection of *Cryptosporidium***.**
    a. Using the above hypothetical case, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **positive?**
    b. In addition, according to your opinion and/or experience, what is the **MINIMUM** number out of these 100 samples that you are 95% sure will be **positive?**

TEST	MINIMUM NUMBER OF POSITIVES	MOST POSSIBLE NUMBER OF POSITIVES
MERIFLUOR		
SAFEPATH		

Example 2 NON-INFECTED SAMPLES CASE:



According to an expert opinion and/or experience, if the test X is applied to 100 known **NON-INFECTED** samples, the **MOST POSSIBLE** number of samples the test will detect as **negative** is 99 (mode, black line), and the expert is 95% sure the **MINIMUM** number of samples the test would detect as **negative** is 85 (red line, percentile 5th)

For the following NEGATIVE SAMPLE cases:

1. In the hypothetical case of applying the test to 100 known *Giardia* **NON-INFECTED** fecal samples from dogs and cats, for the detection of *Giardia* **.**
    a. Using the above hypothetical case, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **negative?**
    b. In addition, according to your opinion and/or experience what is the **MINIMUM** number out of these 100 samples that you are 95% sure will be **negative?**

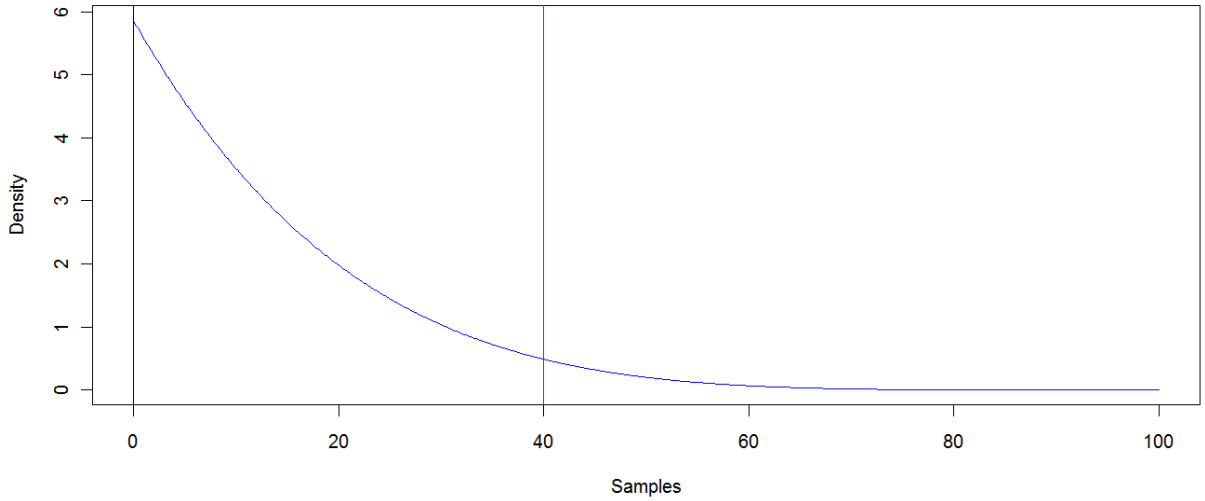
TEST	MINIMUM NUMBER OF NEGATIVES	MOST POSSIBLE NUMBER OF NEGATIVES
MERIFLUOR		
SAFEPATH		

TEST	MINIMUM NUMBER OF NEGATIVES	MOST POSSIBLE NUMBER OF NEGATIVES
ELISA		
SNAP		

2. In the hypothetical case of applying the test to 100 known *Cryptosporidium* **NON-INFECTED** fecal samples from dogs and cats, for the detection of *Cryptosporidium***.**
    a. Using the above hypothetical case, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **negative?**
    b. In addition, according to your opinion and/or experience, what is the **MINIMUM** number out of these 100 samples that you are 95% sure will be **negative?**

TEST	MINIMUM NUMBER OF NEGATIVES	MOST POSSIBLE NUMBER OF NEGATIVES
MERIFLUOR		
SAFEPATH		

Example 3 PROPORTIONS OF INFECTED SAMPLES:



According to an expert opinion and/or experience, if a population X is tested with the aim for detection of the organism Y, the **MOST POSSIBLE** number of samples with the presence of the

organism Y is 0 (mode ,black line), and the expert is 95% sure that the **MAXIMUM** number of samples with the presence of this organism is 40 (percentile 95th, red line).

For the following populations

1. 100 fecal samples from dogs and cats that are submitted to a parasitology laboratory with **LOOSE OR DIARRHEIC STOOLS**.
    a. Using the above hypothetical populations, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **INFECTED with** *Giardia* ***?***
    b. According to your opinion and/or experience, what is the **MAXIMUM** number out of these 100 samples that, you are 95% sure, will be **INFECTED with** *Giardia* ***?***
    c. Using the above hypothetical populations, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **INFECTED with** *Cryptosporidium****?***
    d. According to your opinion and/or experience, what is the **MAXIMUM** number out of these 100 samples that, you are 95% sure, will be **INFECTED with** *Cryptosporidium****?***
    e. Using the above hypothetical populations, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **CO-INFECTED with** *Giardia* **and** *Cryptosporidium****?***
    f. According to your opinion and/or experience, what is the **MAXIMUM** number out of these 100 samples that, you are 95% sure, will be **CO-INFECTED with** *Giardia* **and** *Cryptosporidium****?***

TYPE OF INFECTION	MAXIMUM NUMBER OF SAMPLES INFECTED	MOST POSSIBLE NUMBER OF SAMPLES INFECTED
GIARDIA		
CRYPTOSPORIDIUM		
BOTH		

2. For 100 fecal samples from dogs and cats that are submitted to a parasitology laboratory with **NORMAL TEXTURE.**

a. Using the above hypothetical populations, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **INFECTED with** *Giardia* ***?***

b. According to your opinion and/or experience, what is the **MAXIMUM** number out of these 100 samples that, you are 95% sure, will be **INFECTED with** *Giardia* ***?***

c. Using the above hypothetical populations, according to your opinion and/or experience, what is the **MOST POSSIBLE** number out of these 100 samples that will be **INFECTED with** *Cryptosporidium****?***

d. According to your opinion and/or experience, what is the **MAXIMUM** number out of these 100 samples that, you are 95% sure, will be **INFECTED with** *Cryptosporidium****?***

TYPE OF INFECTION	MAXIMUM NUMBER OF SAMPLES INFECTED	MOST POSSIBLE NUMBER OF SAMPLES INFECTED
GIARDIA		
CRYPTOSPORIDIUM		