A Review on Diagnostic Techniques in Veterinary Helminthlogy

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Abstract: Helminth parasites can be diagnosed based on clinical signs together with history of the animal. However, requires confirmation special laboratory procedure. If an accurate diagnosis cannot be obtained by other methods, but laboratory procedures are used for what assistant they may offer. The detection techniques of these parasites include fecal examination, culture of larvae, molecular and post mortem examination techniques. Faecal examination techniques are qualitative and quantitative techniques. Qualitative faecal examination includes direct smear, sedimentation and flotation technique. Floatation techniques include simple flotation, centrifugal flotation and FLOTAC techniques. The major quantitative techniques include McMaster, Baerman technique, stool egg count and modify Wisconsin sugar flotation methods can be used in parasitic helminth parasite diagnosis. In laboratory and field diagnosis of helminth infections a great variety of methods such as fecal egg count, larvae count and larvae identification are important. Best diagnostic techniques are very important in order to know etiology agent of similar morphology of helminth parasites like Trichostrongyloidea species using other morphological identification such as sheath tail extension. As a conclusion the above diagnostic techniques enable an individual to identify the causative agents of the parasitic disease so that proper treatment, prevention and control measures of helminth parasitic diseases can be established. The objective of this seminar is to review diagnostic techniques which have been employed to diagnosis helminthes parasite and to summarize advantage and disadvantage of each diagnostic technique.

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1. Introduction

Helminth parasites are parasitic worms that feed on a living host to gain nourishment and protection, causing poor nutrient absorption, weakness and disease in the host. Helminth parasites include nematode, trematode and cestode. These parasites feed on the tissue or body fluid or competing directly animal food (Solusby, 2007).

Diagnosis of Helminth parasites is important to prevent the constraint of these parasitic infections. It is the process of determining the cause of the disease or how to identify a particular parasite species. Veterinary parasitology as a clinical subject endows the future veterinary specialists with theoretical knowledge and correct practical skill of diagnosis, treatment, therapy and prophylaxis of invasive disease of animals. It is frequently impossible to make correct diagnosis based on clinical signs only because they are similar in majority of helminthiasis that is why helminthiasis are being usually diagnosed by spotting parasitic worms, their fragment, egg and larvae (Hendrix, 2006).

Diagnosis of infection of parasitic both qualitative and quantitative is largely still depend on relatively inaccurate methods such as fecal worm egg count without which no indication can be obtained of the identities of most of the common worm genera, excepting for those genera with morphologically distinct ova, Although some progresses have been made with computerized identification measuring first stage larvae of protostrongylids and third stage of larvae or length of sheath tail extension can aid identification (Chudic and Gupta, 2003).

Generally, control and treatment of disease can be successful only when preceded by accurate diagnostic techniques. In laboratory and field diagnosis of helminth infection, there are a great variety of techniques, such as direct smear, sedimentation, flotation, McMaster, Baermann technique, molecular technique, postmortem examination, fecal egg count, larvae count and pasture larvae count (Biu *et al.,* 2014). Correct diagnostic techniques important to identify parasite species level (Bayon, 2005). Therefore, the objectives of this seminar paper are to review diagnostic techniques, which have been employed to diagnose Helminth parasites and to summarize advantage and disadvantage of each diagnostic technique.

2. Examination of Fecal Samples

The faeces are by far the most important excretion to be used in aid for diagnosis of internal parasitism. The examination of faeces will provide evidence or in some cause an accurate identification of most of the parasite that inhabits the GIT tract as well as certain parasite inhabiting respiratory tract (Kirberger *et al.,* 2007). This examination is wider meaning, the detection of the parasites and their eggs and bacteria from faeces, but generally it refers to the examination of helminthes eggs and oocysts. The detection of helminthes eggs and larvae is the definite proof of adult helminthes. However, certain parasites produce similar eggs oocysts cannot be identified to the species level in many strongyle type of egg from livestock (Minami *et al*., 2001).

The advantage of examination of fecal sample is simple and less costly, and its disadvantage is certain parasites produce similar eggs; oocysts cannot be identified to the spps level limitation of fecal examination in diagnosis of helminth parasites such as the demonstration of parasite eggs or larvae in feces provide evidence the animal is infected but does not indicate degree of infection (Foreyt, 2001).

2.1. Collection of fecal sample

Faeces intended for parasitological examination should be collect from rectum unless the animal includes air by preferable plastic to the act of defecation when the sample may be collected from the ground. The sample should be fresh and cold to prevent the development and hatching of the egg. The process of development and hatching of common strongylid egg can be slowed by refrigeration. The sample must be packed and preserved in an equal volume of 5%-10% buffered formalin for fixation of samples. It is important to have fresh faeces for testing; this fact must be emphasized to clients. If fresh faeces cannot be promptly submitted, clients should be advised to refrigerate the sample for no more than 24 hours. Take sample from rectum of large animals using disposable plastic glove and dropping of pet animals should be collected immediately after defecation. For small animals a thermometer or glass rod may be used (Hendrix, 2006).

2.2. Gross examination of feces

Gross parasite: spontaneously discharge tape worms and nematodes can be recognized by direct microscope inspection of the faeces, or naked eye. Probably the most common are proglottids of tape worms, entire round worm or even larval arthropods. Their morphology, shape, size and movement may aid identification (Kassai, 1999).

Consistency: the condition of faeces; that is weather the faeces are soft, watery (diarrhea) very hard or constipation should be noted. This description will vary with animal species (Shah-fishery, 1989). Color: light gray color faeces-indicates excusive fat in the faeces is a sign of poor intestinal absorption. Unusual fecal colors should always be reported (Hendrix, 2006). Blood: may indicate sever parasitism as well as other intestinal diseases (MAFF, 2006). Mucus: may be associated with intestinal parasitism or some metabolic disease (Urquhart *et al.,* 1996). Age of the faeces; if the faeces appear old and dry this should be noted. An aged samples, parasitic eggs may be embryonated or larvated, Oocyst may have sporulated and pseudo parasite may be present (Bayou, 2005).

2.3. Microscopic examination of faeces

Compound microscopes used in a veterinary diagnostic setting vary widely in feature and in the magnification they provide. For parasitological examination objective lenses magnification power of 4x, 10x, 40x are most often used, oil immersion occasionally used in veterinary practice. Microscopic examination of fecal sample, this techniques allows the magnification of the object a larger and easily to be seen under the microscope. When a parasite egg or cyst is observed at low magnification, higher power objective may be used to more closely examine it. Strongyloidasis and whip worm can be diagnosed by examine stool under microscopic for presence of worm. Each circular area of the slide through the cover slip is called a field. The size of various stages of many parasites is often important for correct identification by calibrating the microscope. Accurate measurement is easily obtained by using calibrated eyepiece on the microscope (Hendrix, 2006).

2.4. Qualitative fecal examination techniques

It is used to know wheatear an animal is infected or not or presence or absence of infection. Common qualitative fecal examination techniques include: Direct smear, Sedimentation and Flotation techniques, which are used for qualitative fecal examination techniques (Zimmer, 2008).

2.4.1. Direct smear

Direct smear used to detect motile parasites stage helminth larvae frequently passed in the semi-formed and loose to fluid feces of animals. The tropozoiet stage of Giardia species and several Trichomonas species can be found in the loose stool of many different host species (MAFF, 2006). It is the simplest method of microscopic fecal examination for parasites, which consists of a small amount of feces placed directly on microscope slide (Bowman *et al*., 2003).

Direct smear is possible to demonstrate the presence of eggs and larvae of helminth in feces by examining of a thin smear on microscope slide under low power objective of compound microscope. In some cause it may be desirable to use a direct smear

for examining fecal material, because the sensitivity of this test is very low not recommended for routine examination. If the fecal layer is too thick it will not be possible to distinguish the movement of trophozoites. This movement is principal diagnosis for trophozoites in fresh fecal sample (Sloss, 1994). This method is used when adequate laboratory facility are lacking (Kassai, 1999).

The advantages of direct smear are the short procedure time and minimal equipment needed. It does not distort eggs or larvae of parasite unlike other methods due to their effect by concentration media. The direct smear allows the diagnostician to observe egg and larvae undistorted by procedure. However, this procedure has its own disadvantage: the amount of fecal sample is so small that it does not represent a good sample size. Therefore, negative findings are inconclusive but positive results are just as valid as those obtained in more efficient concentration techniques. This procedure leaves a lot of fecal debris on the slide, which may confuse with the nematode egg (MAFF, 2006).

2.4.2. Fecal sedimentation

Principle: this technique concentrates eggs in the sediment and is primarily used to detect eggs or cysts that have too high specific gravity to float or these would be severely distort by flotation solution. Sedimentation procedures concentrate both feces and eggs at the bottom of liquid medium, usually water. Sedimentation detects most parasite eggs but it is not as good as flotation for providing a clear sample for microscopic examination. Sedimentation can be used for round worm and tape worm eggs, but there is usually too much fecal debris hiding the eggs to make it worth while (Hendrix, 2006).

It is more suitable for recovering heavy eggs (e.g. Flukes, *Physloptera* species eggs) that do not float well, because of hypertonic effect excreted by flotation solution. The fluke find is an apparatus for performing sedimentation test in laboratory, by using several screens to rapidly remove fecal debris. This device is very useful in practices conducting routine fecal examination for flukes. Sedimentation for fluke eggs is the method of choice for routine FEC when the fluke infections are suspected. Unlike flotation examination, sedimentation test has only limited concentrated ability; more debris can be removed from fecal sample, if centrifugal sedimentation examination is performed (Sloss, 1994).

Simple sedimentation method: In simple sedimentation, tap water is combined with feces and allowed to settle briefly before supernatant is removed. This allows removal of fine particulate materials. This method is used mostly for helminth eggs which do not float well in common salt solution: e.g. *Fasciola* and *Paramphistomum* species. This method is more accurate techniques (Salam *et al*., 2009).

Ethyl acetate sedimentation method: The organic solvent can remove a considerable amount material. Ether is highly flammable and both ether and ethyl acetate should only be used in ventilated area. To test a faecal sample using this method when you remove the centrifuge your tube will have clearly defined layers. Acetyl acetate layer is on the top, plug of dissolved fat in the middle, layer of water and a pellet of sediment at the bottom (Foreyt, 2001).

Advantage: Sedimentation is more sensitive than the direct smear in terms of the number of organisms demonstrates and the slide is easier to read because much of the fecal debris has been removed. Sedimentation is particularly appropriate for trematode and acanthocephalan eggs, (Chaudic and Gupta, 2003). Sedimentation has its greatest advantage in suspected trematode (fluke) infection. Some laboratory increases specific gravity of the flotation to 1.3 to ensure recovery of fluke egg by flotation (Salam *et al.,* 2009). The problem with the use of flotation method for recovery of egg is that the egg may damage by the high concentration of the solution and become hard to identity (Urquhart *et al.,* 1996). Disadvantage: It is less sensitive than the flotation in concentrated sucrose or most trematode eggs and coccidian oocysts including cryptosporidium (Kassai, 1999).

2.4.3. Fecal flotation

Principle: the fecal flotation technique is most commonly used in veterinary medicine for examination of feces, which is based on the principle that the parasite eggs are less dense than the fluid flotation medium. It is the most satisfactory method that involves in separating the eggs from faecal debris by floating them on variety solution. Fecal flotation methods levitate the diagnostic product of endoparasite organisms (eggs, larvae, oocysts and cyst in feces of animal) by using suspension medium with a higher specific gravity than parasitic products. Parasite eggs, cysts and oocysts are concentrated on the surface of medium because of their lighter density and the result is a clean preparation for microscopic examination with a minimal amount of distracting fecal debris (Christie *et al*., 2011).

Faecal flotation procedures are based on differences in specific gravity of parasite eggs, cysts and larvae of fecal debris. Specific gravity refers to the weight of an object or parasite egg compare with the weight an equal volume of pure water. Most parasite eggs have a specific gravity between 1.1 and 1.2 g/ml whereas tap water only slightly higher than 1 g/ml. Therefore parasite egg can float in a liquid with a higher specific gravity than that of the egg has. Such liquids are called flotation solutions and consist

of concentrated or various salts added to water to increase the specific gravity of water. Flotation solutions usually have specific gravity between 1.2 and 1.25. In this range fecal material much of which have specific gravity of 1.3 or greater does not float (Christie, 2011).

Fecal flotation solution: The most common flotation solutions are sheather's solution (sugar solution), saturated sodium chloride, sodium nitrate, magnesium sulfate (Epsom salts) and zinc sulfate (MAFF, 2006). Sheather's solution or sugar solution: less efficient than sodium nitrate solution because it floats fewer eggs and quite sticky; however, it is ready available and inexpensive, does not distort round worm eggs (Bowman *et al*., 2003). Sodium nitrate solution: is the most efficient floatation solution, but it forms crystals and distort the eggs after a time. Sodium nitrate may be difficult to acquire, but it can be purchased through chemical supply houses (Zajac and Conboy, 2012).

Saturated sodium chloride solution: is the least desirable flotation solution. Its main disadvantages are that it corrodes expensive laboratory equipment such as compound microscope and centrifuge and it forms crystals on the microscope slide and severely distort the eggs. Heaver eggs may not float in the solution, inexpensive, easily prepare and readily available (Kassai, 1999). Zinc sulfate solution: similar in efficiency to sugar solution and can be purchased through chemical supply house. Cystic stages of intestinal protozoan such as giardia, best concentrated with zinc sulfate solution with less distortion (Cringoli, 2006). Magnesium sulfate solution: forms on crystal on the microscope slide and it is expensive (Smyth, 1996).

Flotation solution and fecal preservation methods have fundamental role in determining the analytic sensitive, the precision and the accuracy any copro-microscopic techniques either qualitative or quantitative based on flotation including FLOTAC technique. To prepare saturated solutions add the solute to warm water. The higher specific gravity of the flotation solution is the greater the variety of parasite egg that float. Most flotation fluid distorts the shape of the eggs and the Oocyst. Therefore, examine samples as quickly as possible (Bowman *et al.,* 2003).

Table 1: Types of fecal flotation solution

Flotation solution	Solute	Solvent	Specific gravity	Parasites
Saturated salt	305g NaCl	1000ml tap water	1.18-1.2	Trichostrongyloidea, Strongyloidea
Magnesium sulfate	450g $MgSO_4$	1000ml tap water	1.2	Metastrongylus
Sheath solution	454g table sugar	355 tap water	1.27	*Tania* species, Trichuris, Toxocara
Zinc sulfate	331g Zinc sulfate	1000ml tap water	1.18-1.2	Giardia, Ostertagia schist soma trematode
Zinc chloride	336 g Zinc chloride	1000ml tap water	1.3	Cestode and nematode, Oocyst of protozoa
Sodium nitrate	338g Sodium nitrate	1000ml tap water	1.18-1.2	Spirocercosis

Source: (Dryden *et al*., 2005).

Zinc sulfate centrifugation procedure is the gold standard for the diagnosis of *Giardia* species in dogs. Centrifugation consistently recovers more eggs than other techniques even when as a 5 min centrifugation with a 20 min simple flotation (Zajac *et al*., 2002).

Advantage of flotation technique is to produce clear material than sedimentation for lighter egg amount. It is easy and inexpensive to perform. Flotation techniques are effectively used for demonstration of cestode (except *Diphyllobothrium* species) and nematode eggs. Heavier eggs float well only in salt solution of higher specific gravity. However, this technique has its own disadvantage; the well of the egg and cysts will often collapse thus hindering identification and also some parasite eggs do not float (Shapiro, 2004).

Simple flotation: It is based on the separating of the eggs from faecal material through concentrating them by means of a floatable fluid with an appropriate specific gravity (Rinaldi *et al.,* 2011). Principle: The basis of any flotation is that when worm eggs are suspended in a liquid with a specific gravity higher than that of the eggs; the latter will float up to the surface. Parasite eggs are too heavy to float in tap water. To make the eggs float, a liquid with a higher specific gravity than that of the egg must be use such fluid is called flotation fluid. If specific gravity is above 1.25, add water until the proper reading is obtained. But the problem of this technique is that it

fails to float some trematode eggs. It is probably the second most common parasitological test performed in veterinary practice after direct smear. This method is less efficient than centrifugal flotation, but it does not require a centrifuge (MAFF, 2006).

Modified Wisconsin sugar flotation method: This method is used for determining the egg per gram of feces and it is probably the most commonly used method. It is the most accurate as it counts all the eggs in 3 gram of feces and because it is a flotation method. It has little debris to interfere with the count. It gives quantitative result or used for counting purpose (Cox and Todd, 1992).

Centrifugal fecal flotation technique: It has been advocate as an important aid to flotation for microscopic examination. The procedure more efficiently recovers parasite eggs and cysts and it requires less time than simple flotation. In this technique, fecal debris has settled more rapidly in the centrifugal fecal flotation than standing fecal flotation. Centrifugation is especially necessary when the flotation procedure is performed using viscous solution like Sheather's sucrose. It is the most reliable method of recovering ova and cyst of GIT parasites, including tape worm and whip worm (Zajac *et al*., 2002).

3. Quantative Fecal Examination Techniques

Quantitative procedures indicate the number of egg or cyst in the hosts present in each gram of feces. The results of these procedures are rough or, approximately, indication of the number of adult parasite present with in the host. The procedure is not completely accurate because different species of parasites produce different number of eggs. Several procedures used to estimate the number of parasite eggs or cysts per gram of feces, including the McMaster technique, modified wiscon sugar flotation, and Stoll egg counting technique (Zajac, 2002).

3.1. McMaster technique

It is used to determine the number of eggs present per gram of feces /EPG/. The McMaster slide consist of two glass or plastic slides join together (by aquarium sement) between the mark areas of the upper and bottom slide two chambers of 0.15 volume each are formed. The chambers are filled with a suspension of feces in the flotation. The nematode and cestode eggs float to immediately below the upper glass of the chamber where they can be readily counted under the microscope, while most fecal debris sinks to the bottom (Nicholls, 1994).

Modified McMaster chamber has been developed for trematode eggs and nematode larvae. This is a standard quantitative egg count method, a dilution modification of the quantification fecal flotation technique; method of choice in equine and food animals (Rinaldi *et al.,* 2011). The McMaster technique is one of universally used for FEC. Some laboratory factors; however, affect the reliability of the McMaster technique; among these the McMaster slide area or volume is very important. The higher the volume is the higher the reliability of the McMaster technique. To get the number of eggs per gram of feces, multiple the number under one chamber area by 100 and two chambers by 50 (Hendrix, 1998).

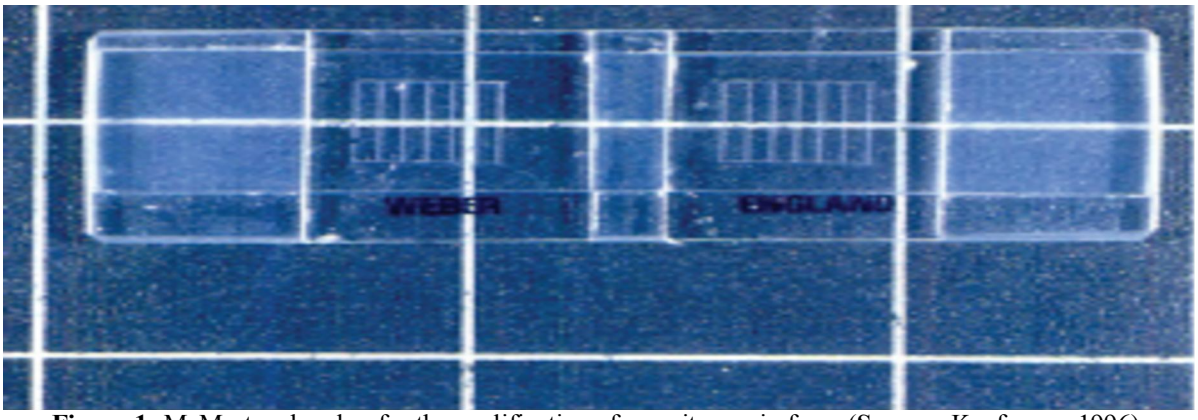

Figure 1: McMaster chamber for the qualification of parasite egg in feces (**Source:** Kaufmann, 1996).

Interpretation: Eggs are less valuable in making judgment about the clinical condition of the individual animal, since many factors affect the accuracy of faecal egg counts. The consistency, the more watery the feces, the more the eggs are diluted, Species of the parasite their egg production varies depending on the type of the parasite, and Immunity status of the host can be suspended ovulation on the part of the worms, and. New infection: immature worms are unable to produce an egg through in a number of species they are highly pathogenic (Maximovi, 2002). Therefore, the EPG result should be interpreted carefully; however, it is generally considered that, if more than 1000 EPG is heavy

infection, if more than 500 EPG is moderate infection and if less than 500 EPG is low infection (Shah-fisher, 1989).

Table 2: Interpretation of EPG in sheep and cattle

Helminths	EPG levels indicative of intermediate degree of infection	
Mixed infection with unspecified GIT nematodes	Sheep 1000-2000	Cattle 200-700
Oesophagotomum	1000-2000	200-700
Bunostomum		50-100
Haemonchus	2500-800	200-700
Ostertagia, Trichostrongyle	250-2000	100-500
Nematodirus	100-600	
Fasciola	200-500	10-25

(Source; Kassai, 1999).

3.2. Baerman technique

Baerman examination is used to isolate larvae from fecal samples and is used mostly to diagnosis lung worm infections. It is used to recover the larvae of nematodes from feces, soils or animal tissues. Principle: The barman technique is used to isolate lung warm larvae from fecal samples and infective larvae from fecal cultures. It is based on the active migration of larvae from feces suspended in water their subsequent collection and identification (Taylor and Coop, 2007).

There are helminthes larvae rather than eggs within the host's fresh feces for example in lung worm of ruminants, L1 of many other nematode species develop rapidly and hatch shortly after feces collected: when identification of genera of species of GIT nematodes is required, L3 are cultured in fecal preparations. If feces containing living larvae are placed in to water, larvae migrate into the water and then sediment. If an accurately weighted amount of feces used, the method is suitable for quantitative assessment to determine the larvae per gram of feces (Kahn, 2006).

It is very important if the fecal be fresh. If feces of grazing animal are being examined and an old sample is used, *trichostrongyle*, strongyle or *Strongyloid*es eggs may have hatched, or free living nematodes may have invade the sample.. If feces are fresh barman method is used for separating lung warm larvae. If feces are not fresh the flotation procedure should be used (Bowman *et al.,* 2003).

Baermann method is based on thermotropism of larvae due to it the larvae of parasites leave feces, penetrate through a sieve settle in rubber pipe. Baerman apparatus consist of support or holder, funnel of the middle side insert into support, rubber pips, clamps for clamping the free end of rubber pipe, and metal sieve (Sloss, 1994).

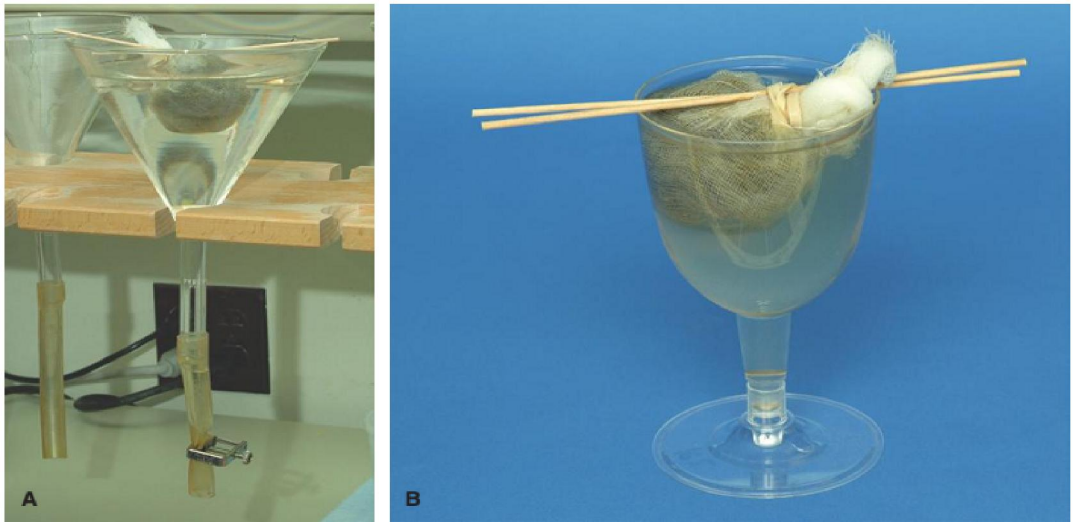

Figure 2: (A)The traditional Baermann apparatus consisting of suspended funnel with clamped tubing attached. For diagnostic testing of fecal sample; it is more convient to perform the Baermann a disposable plastic wine glass (B) **(Source**: Zajac and Cowboy, 2012).

3.3. FLOTAC technique

FLOTAC is a multivalent sensitive and accurate copromicroscopic method of examining fecal samples for the presence of eggs, larvae, oocysts and cysts. This technique uses the novel FLOTAC apparatus which allows up to 1gm of faces to be prepared for microscopic analysis and it is designed for a multivalent fecal egg count (Cringoli, 2006). It is suitable to estimate the prevalence and intensity of infections for epidemiological survey. It is based on flotation in centrifuge and translation of apical portion of the flotation suspension, and gives eggs, larvae, oocyst counts in quantities of feces up to one gram. FLOTAC apparatus is also very useful in order to recover parasitic elements after flotation (Cringoli, 2006).

FLOTAC technique is the traditional flotation method cannot guarantee that the total quantities of parasitic elements present in the sample float to the top of the suspension, and that the entire floated parasitic element adheres to the underside of the cover slip. FLOTAC technique takes advantage of the fact that when flotation takes place in a centrifuge. All parasitic elements float to the top, and if the top portion of the flotation suspension is transversely cut (translated), all parasitic elements can be collected (Rinaldi *et al.,* 2011).

It is cylindrical shaped device composed of three physical components: the body, the translation and the reading disc. The novel aspect of the FLOTAC technique is based on flotation in centrifuge and translation of the apical portion of the float suspension permits the simultaneous coprological diagnosis of several helminths and protozoa. For example, in the diagnosis of sheep and goat parasites, the use of the FLOTAC apparatus combined with the use of two flotation solution (one at low density in one chamber and one at high density in the other chamber of the FLOTAC) permits the detection and counts of the elements of different parasites such as oocysts of *Eimeria*, egg of gastrointestinal nematode species; *Strongyloides* spp, *Trichuris* spp, trematodes, lungworms (Rinaldi, 2011).

The FLOTAC technique is particularly suitable for situations of low parasite EPG/LPG/OPG because other less sensitive techniques often give false negative results. Another source of high reliability is the FLOTAC itself. It is very easy to read the ruled grids because of their clarity, brightness, and precision and the volume of its two flotation chambers firmly and accurately held to 10 ml. In addition, it permits the recovery of parasitic elements after flotation and others (Cringoli, 2006).

3.4. Stoll egg counting method

The stool egg counting technique is a method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. The advantage of this technique is that it requires no specialized equipment. The disadvantage is that the counting takes along time because of the amount of extra or non egg material on the egg. The result obtained by this method is as valid as that of McMaster egg counting technique. This is a simple dilution procedure which facilitates the recognition of eggs and larvae and permits of a quantitative determination of their concentration in the feces. The removal of fine particle and coloring from the medium makes the examination easier, quicker, and more accurate (Hendrix, 2006).

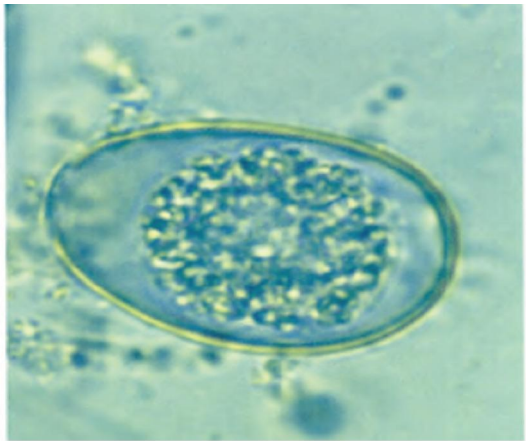

Figure 3: Oocyst of *Eimeria bovis (*26-32µm) x (18-28µm) (**Source:** Kaufman, 1996).

3.5. Fecal culture

Fecal culture is used to differentiate parasites whose eggs and cysts cannot be differentiated by examination of fecal sample. It is a method of incubating eggs to hatch so that the larvae hatched can be identified according to the criteria given in keys. For example the eggs of large strongyles in horse are very similar to those small strongyles. To distinguish between them, feces containing strongyle eggs should be allowed to incubate at room temperature for several days while larvae hatch from eggs. The newly hatched larvae can then be identified. It is generally held that identification of the parasite genera and species in living host animal necessitates the development of third larvae (L3) in fecal cultures by providing a suitable environment (moisture) (Bowman *et al.,* 2003). Isolating *strongyloi* and *Trichostrongyloidea* larvae can be stored and maintain in a shallow layer of water float 4^{o}c -10^{o}c for about 12 month. The similarity in size and appearance of different species of GIT nematode

are such that their differentiation is extremely difficult (Urquhart *et al.,* 1996).

3.5.1. Larvae identification and count

Larvae recovery is isolation of infective larvae could be isolated from fecal sample, fecal culture, and pasture. Larvae best recovered using Baermann apparatus, which is prepared for use, the culture is removed from the incubator and the feces tipped out into the screen of apparatus (Ballweber, 2011). Principle: larva culture is a qualitative and semi-quantitative procedure for isolating, counting, and identifying L3 from representative samples of feces or herbage taken from defined grazing areas. This procedure allows the estimation of larvae availability on the pasture and can be used to defined larvae seasonality and distribution (Kassai, 1999).

Morphological identification of L3 of most parasitic nematode is based on examination of the caudal and cranial extremities, although other features such as the length or shape of esophagus or cranial tip and length of sheath tail extension. Larvae identification: the tip of cranial extremity of larvae is referred to as its head and caudal tip as its tail (Chaudic and Gupta, 2003).

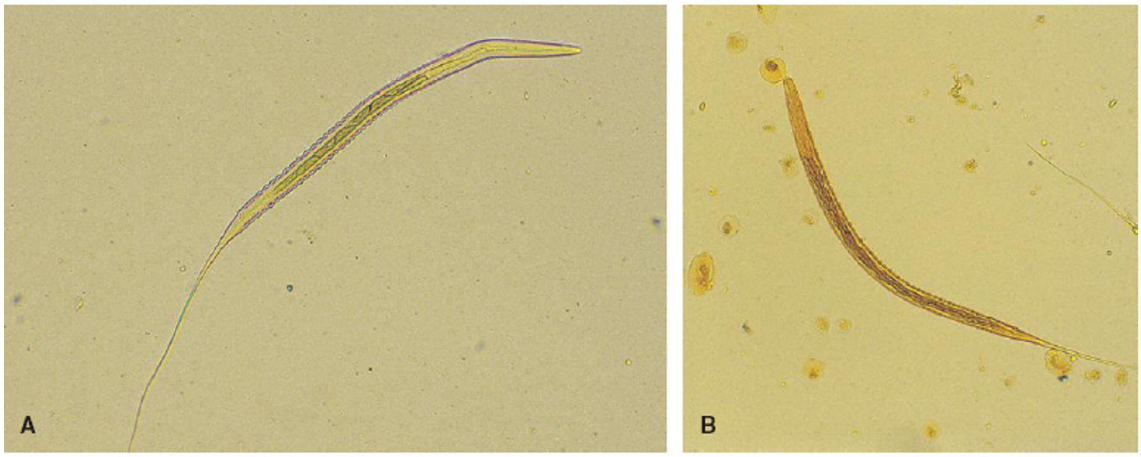

Figure 4: The infective L3 of both large and small strongyle have Avery long filamentous extension of the sheath. Noted A, The larvae of small strongyle (*cyathostomes*).B, the larvae of large strongyle *(Strongylus Vulgaris*) (**Source:** Zajac and Conboy, 2012).

4. Molecular Technique

Molecular techniques have gained increase significance in the detection of parasitic infection even under field condition and sample collected in the field condition over long distance to the laboratory (Kaufmann, 1996).

PCR based diagnosis is advantage of molecular technique: greater potential with regard to specificity and sensitivity, relatively expensive and technical disadvantage demanding for use in developing countries. Some of the techniques are prone to problem of reproducibility often require high degree of skill for consistency and proper interpretation of the result (Wilson, 2005). The detection of various genetic markers for different parasites found in feces is now being routinely done in case of several protozoa (Rollinson and Blackwell, 1999). Molecular technique studying the DNA of the parasite in order to identify it, PCR and PCR linked restriction fragment length polymorphism are used to detect and amplify parasite DNA found in the feces of animal. This technique is very sensitive, which is useful for diagnosing parasites even small number. It is the detection of various genetic markers for different parasites found in feces is now being routinely done in case of several protozoa. The most commonly used are currently assay for cryptosporidium and girdia (Bowman *et al.,* 2003).

5. Postmortem Examination Technique

The PME technique is used for identifying parasites in the GIT, but it may be adapted for the estimation of the number of parasites in GIT and level of Helminth infection, as well as on its pathological consequences, Since, fecal egg and larvae count do not always give a reliable indication of worm burden (Hendrix, 1998; Kassi, 1999). When parasites are recovered from the digestive tract or other parts of the body, it may be necessary to preserve them for identification later and placed 70% ethyl alcohol (Foreyt, 2001).

Postmortem examination technique enables to (detect and count worm), identifying the larval stages, differentiate the species of parasite and determine the epidemiological pattern. Postmortem examination is extremely valuable because of it provides an opportunity to examine everything both inside and outside. Necropsy the most direct and clear information on kind and level of helminthes

infection, therefore the field veterinarian should insist on obtaining whenever. This is conducted on autopsy of corpses of dead and killed, with diagnostical purpose, domestic animals and poultry and identification of helminthes and characteristic patho anatomical changes in organs and tissues (Maximov, 2002).

Conclution and Recommendations

In conclusion the helminth parasites have major effect on livestock production, reduced the productivity of animals, so to prevent their effects on the live animal accurate diagnosis, treatment and prevention methods are necessary. The diagnosis of parasitic disease involved the consideration of their epidemiology, clinical sign and diagnostic techniques. These techniques; qualitative examination techniques: direct smear, sedimentation and flotation. Quantitative examination techniques: McMaster, Baermann, stool egg counting and FLOTAC techniques. Molecular techniques (PCR) and larvae culture for isolation and identification of parasite. Epidemiological studies have to be performed to determine the best type of monitoring and which diagnostic test has to be used. The cost of test should be low enough for farmer and veterinarians to accept it as a tool. Furthermore, veterinarians and farmers will need to be train in the interpretionof the test to get it accepted. Diagnostic techniques for to identifying the etiology of parasites enables in the treatment, prevention and control of helminth parasitic disease. Thus treatment for a given parasitic disease is sated up after proper and definitive diagnosis of the causal agent.

Based on the above conclusion the following recommendations are forwarded:

- Since parasitic diseases are not easily diagnosed by only considering the clinical signs, equipped diagnostic techniques should be established.
- The laboratory should fulfill necessary equipments, reagents and skilled man power.
- Further research and new technology should be conducted on the diagnosis of Helminth parasites.

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