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Review on Internal Parasitic Diseases of Sheep and Laboratory Based Diagnostic Approaches

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Abstract: Diverse range of internal parasite affects sheep (Ovine). The majority of adult helminthes affecting sheep are grossly visible to the naked eye due to their size. With internal parasites, however, observation and detection of adult stages is generally only possible on post-mortem examination of the appropriate organs and viscera. More often, the presence of parasites in the gastrointestinal tract, lungs and liver can be detected by parasitological examinations of appropriate samples, usually faeces, for the presence of their eggs, cysts or larval stages. This review focuses on the clinical and laboratory diagnostic approaches to a number of important parasitic diseases of sheep, in particular, parasitic gastroenteritis and the detection of species showing the presence of anthelmintic resistance, as well as other diseases, such as liver fluke and coccidiosis. However, misidentification can occur without appropriate experience in parasite identification. Accurate and correct diagnosis is fundamental to good parasite control, otherwise inappropriate or consequential, apparent treatment failures may occur.
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1. Introduction

Sheep can be parasitized by a diverse range of parasites, with well over 150 species of parasites reported worldwide. The majority of helminthes affecting sheep are grossly visible to the naked eye. With internal parasites, however, visual detection of adult stages is generally only possible on post-mortem inspection of the appropriate organs and viscera. The focus of the review will be on the clinical and para-clinical laboratory diagnostic approaches to the detection of parasitism in sheep, rather than on postmortem parasitological examinations. The most important endoparasitic disease seen in sheep, is parasitic gastroenteritis (PGE), which is caused by a range of gastrointestinal (GI) nematodes, as it has a significant cost for sheep farming (West *et al.,* 2009). Given its importance, and the emergence of nematode species showing increasing levels of resistance to one or more of the available anthelmintic groups a large part of this review will focus on the parasitological techniques used in detecting and identifying the presence of pathogenic worm burdens though faecal sampling, as well as on the methods employed to determine their resistance status to anthelmintics. The diagnosis of other important endoparasitic diseases found in sheep will also be reviewed. These include fasciolosis caused by the liver fluke, *Fasciola hepatica*; coccidiosis and cryptosporidiosis, caused by protozoan parasites of the genus *Eimeria* and *Cryptoporidium*, respectively. Other internal parasitic infections seen in sheep are generally of lesser importance and will not be discussed further in this review. These include adult tapeworms (Moniezia); several intermediate stages (metacestodes) of tapeworms (Echinococcus spp., *Taenia spp.*); lungworms (*Dictyocaulus, Muellerius*, *Protostrongylus, Cystocaulus*) and 'nasal bots' (Papadopoulos, 2008).

Therefore, the objectives of this review paper are:

- To highlight laboratory based diagnosis used as a technique to examine sheep for internal parasite
- To review effective laboratory approaches on internal parasite of sheep

2. Gastrointestinal parasitism

Diagnosis of per gram of feces (PGE) is generally based on clinical signs, seasonal occurrence of disease and, where possible, supported by post-

mortem examination and worm burden enumeration. Most species of nematodes affecting the digestive tract cause diarrhoea. In contrast, acute haemonchosis (Haemonchus contortus) is characterised by anaemia, variable degrees of oedema (submandibular oedema and ascites are the forms more easily recognized), lethargy, dark coloured faeces and sudden death (Taylor *et al.,* 2007). Diarrhoea is not generally a feature. Pallor of the mucous membranes is striking and can be assessed by inspection of the conjunctivae using the FAMACHA© assessment system (Kaplan *et al.,* 2004; Bath and van Wyk, 2009), rather than the oral mucosa or skin where differentiation from a normal appearance is difficult. Faecal Occult Blood testing as a means of predicting the severity of *H. contortus* infections has also been used (Colditz and LeJambre, 2008). This utilizes a dipstick type of approach and uses the fact that blood can be detected in host faeces, as a result of worm feeding activity before there is a significant rise in faecal egg counts (FEC). A fluorescent microscopy technique for the differentiation of *H. contortus* eggs from other species, using the lectin binding characteristics of nematode eggs has also been reported. The laboratory-based technique uses a fluoroscein isothiocyanate (FITC)-labelled peanut agglutinin (PNA) lectin. Lectin binding exhibits a genus specific pattern, with Haemonchus spp. staining strongly positive with PNA. Faecal consistency and appearance also provide clues regarding possible species identity and presence. Pelleted faecal samples with moderate to high FEC are generally indicative of *H. contortus* infections. Dark, foul-smelling, diarrhoeic faeces are rather suggestive of *Trichostrongylus* infections. Faecal egg counts are a useful aid to diagnosis, although faecal cultures are necessary for generic identification of larvae and are described in more detail below (Colditz *et al.,* 2002).

2.1. Monitoring of faecal egg counts

Monitoring of faecal egg counts (FEC) can be undertaken in a suitably equipped and trained veterinary practice or via a commercial laboratory.Anon-farm approach is also available in some countries using the FECPAK system (Colditz *et al.,* 2002).

2.1.1. Collection of faeces

Sheep may be sampled individually or as a group, to determine a mean FEC. Fresh dung samples should be collected either from the pasture or alternatively directly from the rectum. At least ten sheep in a group should be sampled. The wide variation in FEC between sheep grazing together in the same field means that random sampling effects have a significant impact on the confidence limits surrounding the estimate of the group mean FEC. Samples should be fresh when collected and kept cool (not frozen) in an airtight container or plastic bag, before delivery to the laboratory within 48 h. If faeces are too old, some eggs will have hatched and the reported egg count will be an underestimate (Abbott *et al.,* 2004, 2007).

2.1.2. Faecal egg counts

Described FEC or coproscopic methods are either qualitative or quantitative. Qualitative methods provide information on the species present, whereas quantitative methods provide an indication of the levels of infections. Both have their own importance in determining the health status of a flock and determining appropriate treatments and control measures. Examination of faeces for helminth eggs may vary from a simple direct smear to more complex methods involving centrifugation and the use of flotation fluids (MAFF, 1986). Flotation methods involve separating the eggs from faecal debris using a variety of flotation solutions with specific gravities, such that worm eggs float to the surface of the suspension. Nematode and cestode eggs float in a liquid with a specific gravity between 1.10 and 1.20; trematode eggs, which are much heavier, require a specific gravity of 1.30–1.35. The flotation solutions used for nematode and cestode ova are mainly based on sodium chloride (NaCl) or sometimes magnesium sulphate (MgSO4). A saturated solution of these is prepared and stored for a few days and the specific gravity checked prior to usage. The standard quantitative technique and the one most widely used is the McMaster method, of which there are various modifications reported in the literature. Reported methods differ in the weight of faeces examined, in the flotation solution used (chemical salt, level of saturation and volume), in the flotation time, in the presence or absence of a centrifugation step, in the design and number of McMaster counting chambers, in the counting method and multiplication factors employed and in whether any correction factors are used to allow for faecal consistency (Dunn and Keymer, 1986; MAFF, 1986). Quantitative FEC results are normally expressed as egg per gram (epg) of faeces. Problems can however, occur when analyzing and comparing the results obtained by different laboratories on the same samples. Therefore, there is a need to provide some degree of standardisation of the numerous modifications of the McMaster method, particularly where these are used in the determination of the presence of anthelmintic resistance by using the Faecal Egg Count Reduction Test (Taylor *et al.,* 2007).

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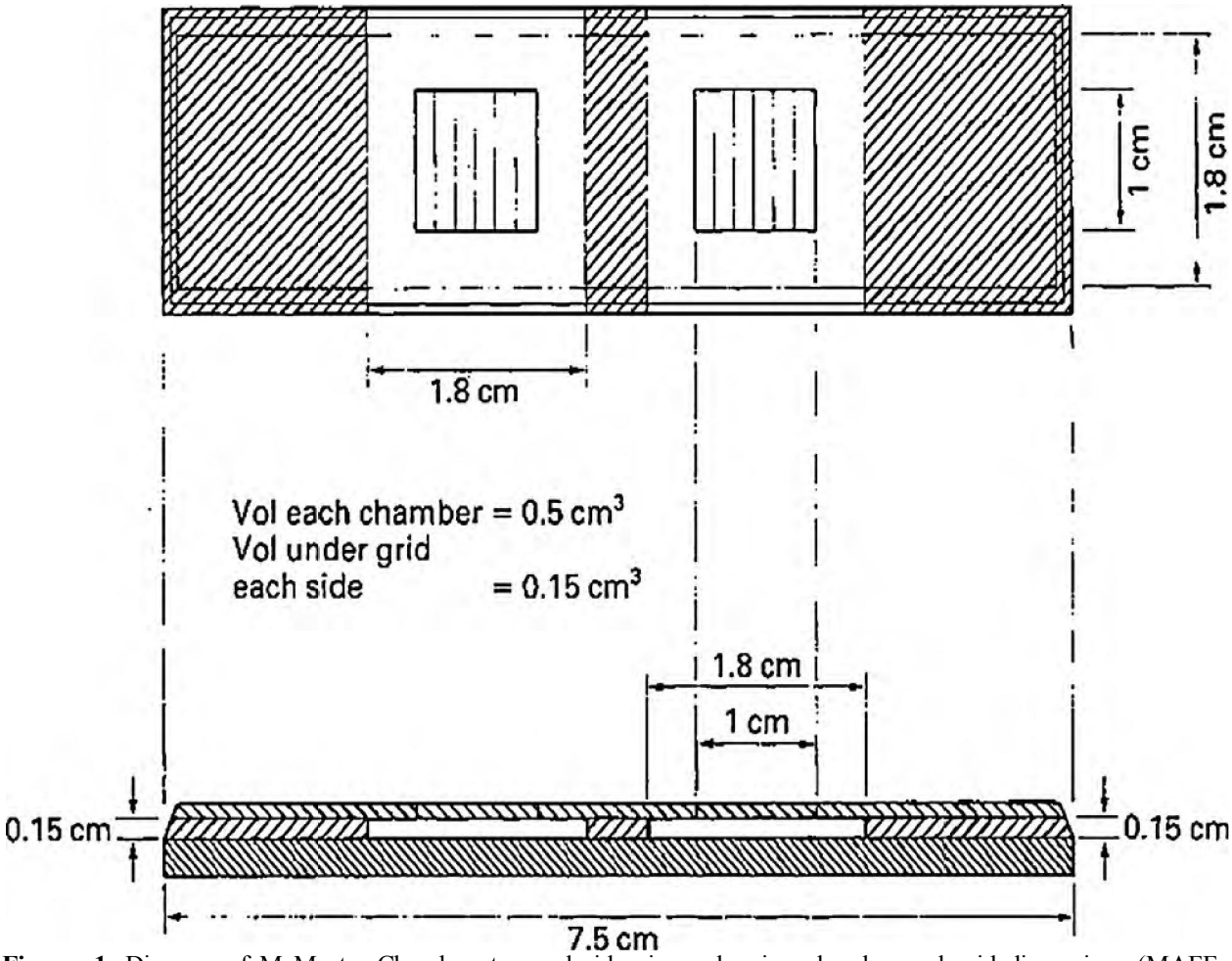

Figure. 1. Diagram of McMaster Chamber: top and side views showing chamber and grid dimensions (MAFF, 1986).

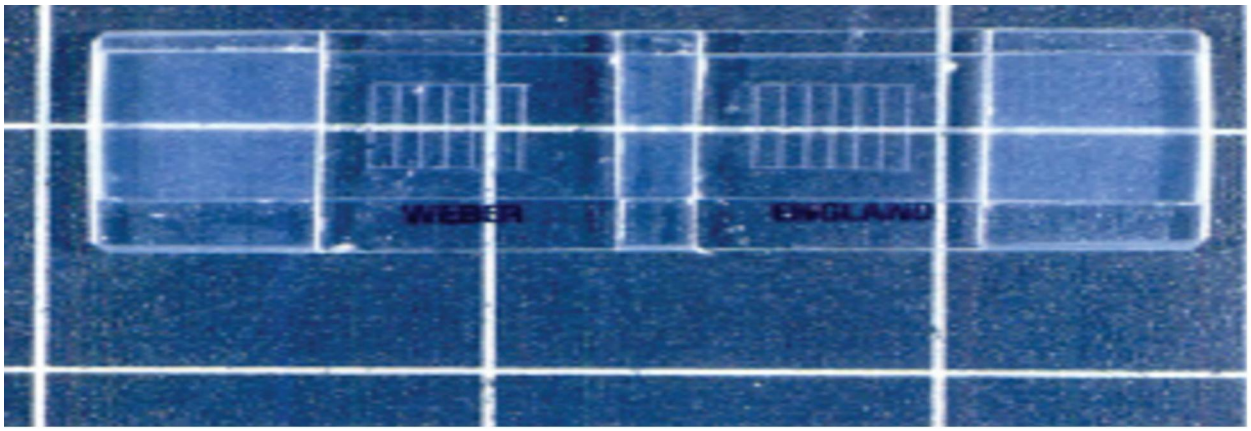

Figure 2: McMaster chamber for the qualification of parasite egg in feces (**Source:** Kaufmann, 1996).

2.1.3. The McMaster Chamber

The McMaster Chamber is the piece of equipment most frequently used in methods for estimating nematode FEC (Fig. 1). It is possible to estimate the number of eggs using different multiplication factors with any one method depending on the dilution of the faeces and the area of the chamber examined. Since both the ruled grids and each chamber are precise measurements, eggs can be counted under one or both grids, under one chamber or under the total area of both chambers (Fig. 1). Thus, for example, where the dilution of faeces is 1 in 15 (3 g in 42 ml of fluids) and the volume under one grid is 0.15 ml, the examination of one or both grids would require a multiplication factor of either 50 or 100, depending on the number of grids examined. In use, the suspension of faeces to be examined is run into each chamber until it is full; then, the chosen area is examined and all eggs seen are counted. The eggs float to lie immediately below the upper glass of the chamber, while the debris sinks to the floor of the chamber. The eggs are therefore clearly in focus, while the debris is quite indistinct (Fig. 2.) (MAFF, 1986).

2.1.4. Interpreting FEC results

It is impossible to calculate from the epg of faeces the actual worm population of the host, since many factors influence egg production of worms and the number of eggs also varies with the species. With some genera (e.g. *Nematodirus*), egg production is not strongly related to the size of the worm burden; with others (e.g. *Teladorsagi*a), the fecundity of adult female worm is inversely density dependent, i.e. egg production per worm is higher when the number of worms in the gut is lower. Nematode genera and species within genera differ in their fecundity and pathogenicity. *H. contortus*, for example, is very pathogenic and is also highly fecund. Tolerable burdens of Haemonchus may produce FECs, which would be considered dangerously high for *Trichostrongylus*. As sheep grow older, they develop an immunity that reduces worm fecundity, so egg count becomes a less reliable indicator of the size of a worm burden. FECs should therefore be viewed as 'supportive diagnostic information' to be considered with history and clinical signs (Abbott *et al*., 2004, 2007). FECs over 1000 epg of faeces are generally considered indicative of heavy infections and those over 500 epg of moderate infection. Careful interpretation is particularly important, where the FEC is low. Low epg is not necessarily indicative of very low infections, since patency may just be newly established; alternatively, the epgmaybe affected by developing immunity. The eggs of some species, such as Strongyloides, Trichuris and Capillaria, can be easily recognized morphologically (Taylor *et al.,* 2007). However, with the exception of Nematodirus spp., the common trichostrongyle eggs require either measurement for differentiation or, more usually, larval culture and identification of infective 3rd stage larvae. Despite these limitations, FECs can be used to help decide if anthelmintic treatment is necessary or can be safely delayed or omitted.

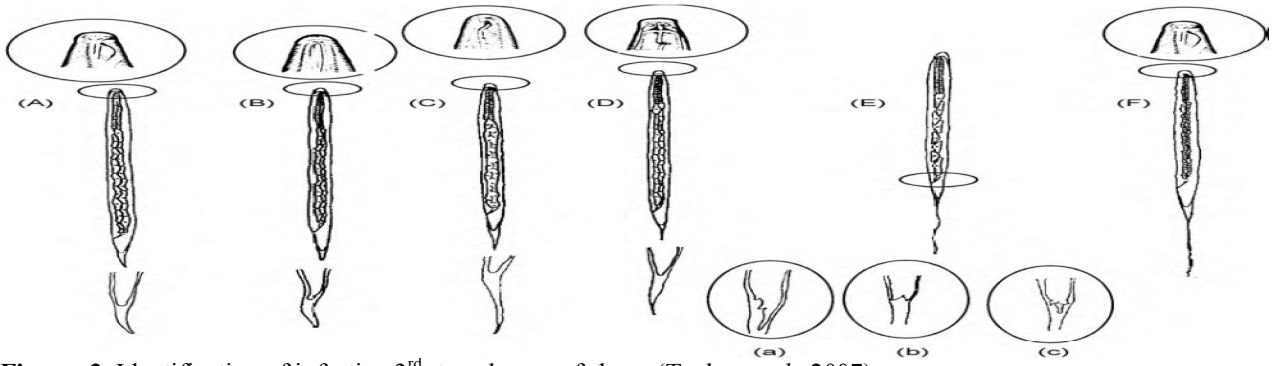

Figure. 3. Identification of infective 3rd stage larvae of sheep (Taylor *et al.,* 2007).
(A) Telodorsagia circumcincta. (B) Trichostrongylus spp. (C) Haemonchus contortus. (D) Cooperia spp. (E) Nematodirus (a) battus, (b) filicollis, (c) spathiger. (F) Oesophagostomum spp. (inserts show differing morphology of larval heads and tails – Nematodirus spp.). Sheep following after treatment depending on the anthelmintic used: it should be 7 days after levamisole administration (LV), 10–14 days after benzimidazole (BZ) administration and 14–16 days after a macrocylic lactone (ML) administration. In practice, this means checking 7 days after administration for LV resistance and/or 14 days after administration for BZ and ML resistance. The test is merely an indicator of anthelmintic inefficacy and not necessarily of anthelmintic resistance per se, as many other factors can influence test results (Abbott *et al*., 2004, 2007).

2.3.2. Faecal egg count reduction tests

A more structured on-farm test can be conducted, in which a number of different anthelmintics is tested against a control. Fifteen to 20 sheep are randomly allocated to control or treatment groups, which might include animals given a BZ, a LV or a ML. FECs are performed prior to treatment on samples from at least ten of the control sheep; then they are performed on samples from the sheep administered with LV 7 days later and then, 14 days after treatment, they are performed on samples from all control sheep and sheep given BZ or ML (Taylor *et al.,* 2002; Abbott *et al.,* 2007). AR is suspected, if the percentage reduction in FEC of a test group compared with those of the control group is less than 95%. Results may differ according to whether arithmetic or geometric means are used in the calculations (Dobson *et al.,* 2008).

2.3.3. Larval development tests

A range of in vitro tests has been developed, to avoid the use of animals in testing for resistance. The two most commonly used are the egg hatch assay (EHA) for the BZ anthelmintics and the larval development test (LDT) for BZ and LV anthelmintics. There are no in vitro tests yet available to test ML resistance. Farm visits are not necessarily required and the samples can be sent by post-directly to the laboratory. However, currently these tests are relatively expensive, precluding their widespread use. Nevertheless, their sensitivity is generally considered to be higher than that of the FECR; thus, AR may be detected when the frequency of resistant alleles within the worm populations is still low. Interpretation is, however, not straightforward and requires expert input (Taylor *et al*., 2002).

3. Liver fluke (fasciolosis)

Diagnosis of liver fluke is based primarily on clinical signs, seasonal occurrence, prevailing weather patterns and a previous history of disease on the farm. Diagnosis should present few problems, especially when postmortem examination can be performed. Clinical signs of fasciolosis vary according to the numbers of mature and immature parasites present and are associated with the level and extent of liver damage. In acute disease, associated with large numbers of immature fluke, affected animals are weak, anaemic and dyspnoeic or may present as sudden death. Chronic fasciolosis is characterised by progressive loss of condition, reduced appetite and development of anaemia, oedema and ascites (Taylor *et al.,* 2007). During the chronic phase, when predominantly adult fluke are present, Fasciola eggs can be demonstrated in the faeces. The standard McMaster technique will not demonstrate trematode eggs, which have a high specific density. For these, a flotation fluid of higher specific gravity, such as a saturated solution of zinc chloride (ZNCl2) or zinc sulphate (ZNSO4) are widely used or, alternatively, a sedimentation method (MAFF, 1986). Two biochemical tests can be also used to determine levels of liver damage. Blood glutamate dehydrogenase (GLDH) increases within the first few weeks of infection, due to the liver parenchymal damage, whilst glutamyl-transpeptidae (GGT) increases following the bile duct damage (Taylor *et al.,* 2007).

4. Coccidiosis

The diagnosis of coccidiosis should be based on clinical signs, farm history, supported where possible by pathological and parasitological findings. Affected lambs are weak, unthrifty and often show faecal staining around the perineum and the hind legs. As disease progresses, some lambs show profuse watery diarrhoea, which often contains streaks of blood (Taylor, 1995; *Taylor and Catchpole, 1994).* Age, parity, stocking density and other predisposing management factors which greatly increase levels of infection and exposure to coccidial oocysts, should also be considered in any presumptive diagnosis. Oocyst counts alone are of limited value, unless speciation is performed. The McMaster flotation method is the simplest technique for detecting the presence and for estimating the number of coccidial oocysts in faeces. The technique is exactly the same as that described for helminthological diagnosis, although the small size of the oocysts makes the microscopic examination more prolonged. If the animal has acute clinical signs of coccidiosis, such as blood-stained faeces, and many thousands of oocysts are present, one may reasonably consider that the diagnosis is confirmed. Unfortunately, with the more pathogenic species of coccidia, clinical signs may appear during the asexual phase or when oocyst production has just started, so that a negative or low oocyst count does not necessarily indicate that the clinical diagnosis was wrong (MAFF, 1986).

5. Cryptosporidiosis

The diagnosis of suspected Cryptosporidium infection depends on the examination of faecal smears stained by the Ziehl-Nielsen technique, the small thin-shelled oocysts appearing bright red. For clinical infections, where large numbers of oocysts are present, this method is sufficiently sensitive for

confirmatory diagnosis. Serological or molecular-based assays are less effective in sheep and generally not appropriate, unless for epidemiological studies (Taylor *et al*., 2007).

6. Conclusion and Recommendations

In conclusion the helminthes parasites have major effect on sheep 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 helminthes 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 Helminthes parasites.

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