Review on Fasciolosis and Anthelmintic Resistance

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Abstract: This paper is mainly aimed to review the Fasciolosis and its drug resistance development. Fasciolosis is a major disease which imposes direct and indirect economic impact on livestock production, in particularly cattle, sheep, goats and occasionally man. The disease is caused by digenean trematodes of the genus *Fasciola* commonly referred to as liver fluke. The two species most commonly implicated as the etiological agents of Fasciolosis are *Fasciola hepatica* and *F. gigantic. F. hepatica* has a worldwide distribution but, it predominates in temperate zones while *F. gigantica* is found on most continents, primarily in tropical regions. Both F. Hepatica and F. gigantica are transmitted by the snails of the family. The effective treatment Fasciolosis depends on the drug choice. Not all compounds are equally effective against stages of development of parasite in the body due to the development of drug resistance. flukicid resistance was not recognized till the report of Triclabendazole resistance in Australia in 1995. And has since been described in Netherlands, UK and Ireland. Although resistance in flukes has not yet reached the levels present in nematodes, resistance exists for the Triclabendazole and Albendazole. Drug susceptibility or resistance from the field and in the laboratory can detected using the In vivo tests and and In vitro tests. Therefore, it can be concluded that Fasciolosis is an important parasitic disease which hinders the ruminants' production. So it is recommended to control the disease by reducing the snail population or by using effective choice of anthelmintics.

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Introduction

Ethiopia possesses the largest livestock population in Africa. But, the production of livestock that practiced in most of the agro-ecological zone and its contribution either at household or national level is so far been limited compare to its large size. Among the numerous factors that responsible for poor production and productivity of live stocks are animal diseases, age- old traditional management system, absence of developed marketing system and the efficacy of the drug for treatments of the different diseases are considered as the major problems coupled with problem of animals feed and nutrition (Kiflu, 2017).

Among the many animal diseases problem, Fasciolosis is a major disease which imposes direct and indirect economic impact on livestock production, in particularly cattle, sheep, goats and occasionally man (WHO, 2009). Fasciolosis is an economically important parasitic disease of domestic livestock which is called by different local names in various parts of Ethiopia that vary according to the region and language. In Amharic, it is called "Kulkult", "Wadomma", "and Yegubet tile". In Afan Oromo, it is known as "Dadao", "Losha", and "RammoTiruu". In Tigray language, it is termed as "Ifil (Yeneneh *et al*., 2012). The disease is caused by digenean trematodes of the genus *Fasciola* commonly referred to as liver fluke. The two species most commonly implicated as the etiological agents of Fasciolosis are *Fasciola hepatica* and *F. gigantic* (Mas-coma *et al*., 2005).

F. hepatica has a worldwide distribution but, it predominates in temperate zones while *F. gigantica* is found on most continents, primarily in tropical regions. Both *F. Hepatica* and *F. gigantica* are transmitted by the snails of the family Lymnaeidae (Terefe *et al*., 2012). The snails for *Fasciola* are dependent of their close environment (nature of the soil), and of the climatic conditions for survival and multiplication of the intermediate hosts and also for the survival and evolution of larval stages (miracidium, sporocyst, redia, cercaria, and metacerceriae) (Dorchies, 2006). Generally, infection of domestic ruminants with *F. hepatica and F. gigantica* causes significant economic loss estimated at over US $200 million per annum to the worldwide and 600 million animals' infected. And it causes high proportion of mortalities, especially in small ruminants and calves (Mungube *et al*., 2006). Whereas, in Ethiopia about 48.4 million Ethiopian Birr per annum is lost due to the presence of ovine Fasciolosis (Ahmed *et al*., 2007).

The effective treatment Fasciolosis depends on the drug choice, as all treatments are not equally

effective against stages of development of *F. hepatica* in the body due to the development of drug resistance. Triclabendazole resistance was first reported in Australia in 1995 and has since been described in the Netherlands, UK and Ireland. At the same time, there has been a dramatic resurgence of Fasciolosis as a result of climate change and the advent of milder, wetter weather (Mitchell, 2002). Previous finding of (Moll *et al*., 2000) and (Khan, 2017) had showed that the occurrence of Triclabendazole resistance in sheep. In addition, resistance development of Albendazole against *Fasciola* was also reported by many authors (Wood *et al., 1995) and* (Desalew, 2014*)*. Of the great concern is fear of the spread of resistance to Triclabendazole, the main drug used to treat fluke infections because of its high activity against the migrating immature stages and adult fluke and the Albendazole which only affects adult fluke. Although resistance in flukes has not yet reached the levels present in nematodes but, making further study on the efficacy of the drug choice for treatment of *Fasciola* strengthened the prevention and control strategy measure. Hence, this review was under taken with following objectives:

- To review Fasciolosis
- To review the drug resistance development of fasciola

Litreture Review

Etiology

Fasciolosis is parasitic disease which is known as fascioliasis, fasciolasis, distomatosis and liver rot that caused by the two species; *Fasciola hepatica* and *gigantica*. The disease is a plant and food-borne trematode zoonosis (Mas-coma *et al*., 2005). And it is classified as a Neglected Tropical Disease (Gagandeep *et al*., 2013). Is the disease of the bile duct of domestic herbivorous animals, contributes to great economic and health losses in the small ruminant industry in many countries worldwide (Qureshi *et al*., 2012).

Fasciola gigantic is larger than *F. hepatica* and can reach 7.5 cm length. The shape is more of leaf like, the conical anterior end is very short and the shoulder characteristic of *F. hepatica* is barely perceptible. *F. hepatica is a leaf shaped fluke with broader anterior and cone shaped anterior projection. It is grayish brown in color changing to gray when preserved. Generally, the morphological structure of Fasciola species are characterized with a cuticle armed with sharp spines. The young fluke at the time of entry in to the liver is 1 - 2 mm in length and lancet like when it has become fully mature in the bile ducts. It is leaf-shaped gray brown in color and is around 2.5 - 3.5 cm in length and 1 cm in width. The anterior end is conical and marked off by distinct shoulders from the body (*Maximou, 1980).

Figure 1: Morphology of *F.hepatica* and *gigantia*. Source: (Tylor, 2007).

The egg of F. hepatica measures 150 µm by 90 µm in size and also similar in shape to that of F. gigantic. Fasiolca eggs should be distinguished from the eggs of other flukes, especially from the large eggs of Paramphistomum. A characteristic of Fasciola egg is yellow-brown in color, large and oval in shape and it has an indistinct operculum (lid) as well as unsegmented ovum surrounded by many yolk cells where as Paramphistomum eggs have transparent shell, distinct operculum with embryonic clear cells and possess a small knob at their posterior ends (Soulby,1982).

Figure 2: Egg of *Fasciola* species. Source: (WHO, 2009).

Epidemiology

Geographical Distribution

Fasciolosis is considered as important limiting factor for bovine and ovine production. The geographical distribution of *F. hepatica* and *F. gigantic* is determined mainly by the distribution patterns of the snails (Peng, 2009). *F. hepatica*, which is a temperate species, is the mostly important trematode of domestic ruminant and common causes of liver fluke disease in temperate areas of the world. *F. gigantica*, on the other hand, is economically important and widely distributed in tropical countries of Africa and Asia. In Ethiopia, *F. gigantica* is found at altitudes below 1800 meter below sea level while *F. hepatica* is found at altitudes between 1200-2560 meters above sea level. Mixed infection by the two species can be encountered at 1200-1800 meter above sea level (Yilma and Malone, 1998). Climatic condition like, temperature and rainfall affect both the spatial and temporal abundance of snail hosts and the rate of development of fluke eggs and larvae (Maqbool *et al*., 2002). In general, non acidic, low lying swampy areas with slow moving water and irrigated areas are highly suitable for infection to takes place. Snails burrow in to the soil to survive dry periods and release cercaria when free water is present (Radostits *et al*., 2007). *L. truncatula* and *L. natalensis* are the most common intermediate host for *F. Hepatica* and *F. gigantica* respectively. Although, there are many Lymnaea species that play their role in the life cycle of *F. hepatica*, for example; *L. tomentosa* in Australia and New Zeeland. In addition, L. *columella* in North America, Australia and New Zealand, *L. bulimoides* in Southern USA and the Caribbean. Furthermore, *L. humilis* in North America, *L. vector* in Southern America and *L. diaphena* in South America. Other vital Lymnaea species of *F. gigantica* are *L. auricularis* in Europe, USA, Middle East, and Pacific islands, *L. rufescens* and *L. acuminta* in India and Pakistan and *L. rubiginosa* in Malaysia (Sarkari and Ghobakhloo, 2012).

Host range:

The adult fluke is a natural parasite of sheep and cattle but also may infect the horse, goat, camel, lama, elephant, buffalo, dog, rabbit, guinea pig, monkey and man, (Mahrukh, *et al.,2011)*.

2.2.3 Transmission of Fasciolosis:

Once an animal is infected with Fascioliosis, it will pass eggs from the faces of the animals. On entering the water, the eggs hatch into miracidia. The miracidia locate water snails by chemotaxys. Sporocyst produce rediae and rediae may produce second generation rediae. Cercariae emerge from the snails. Encystment of Cercariae on vegetation occurs at the edge of water. Sheep and cattle become infected by ingesting metacerceriae while grazing. People normally catch the disease more often in situations where livestock live in the same general areas where food is grown. Most humans catch it from underwater food plants like watercress. Infection can potentially be avoided by cooking these plants fully before eating them. In some areas, eating these plants raw is relatively customary and Fasciolosis in humans is more common in those areas (Usip *et al*., 2014).

Life cycle of *Faciola*

The eggs of flukes passed in the feces of definite host develop and hatch releasing motile, ciliated miracidium. These takes 9-15 days at optimum temperature of 22˚C - 26˚C (Noyer and Coyle, 2002). And little development occurs below 10˚C (Marquardt, *et al*., 2000). The liberated miracidium has a short life span and must locate a suitable snail within 3 hours otherwise die. Inside the infected snails miracidia develop to sporocysts, rediae stages and cercariae consequently. Subsequently, motile cercariae pass from infected snails. Then, cercariae attach to plants and vegetables where they encyst as infective metacercaria. Under unsuitable circumstances this may take several months (Hardi, 2016).

It takes a minimum of 6 - 7 weeks for completion of the development from miracidium to metacercariae. Infection of snail with one miracidium can produce over 600 metacercariae. Metacercaria infestes the final host and encysted in the small intestine, migrate through the gut wall, cross the peritoneum and penetrate the liver capsule. The young flukes tunnel through the liver parenchyma for 6 - 8 weeks and then enter to the bile duct where they migrate to the large ducts and occasionally the gall bladder. Finally, the mature flukes lay eggs and reach the small intestine with bile and ultimately with feces to the environment, where another life cycle begins (Aksoy and Kerimoglu, 2005).

The prepatent period of *F. hepatica* is 10-12 weeks and one entire life cycle of *F. hepatica* may be completed in a minimum of 17-18 weeks. *F.hepatica* may live for years in untreated sheep but in cattle it is usually less than one year. For *F.gigantica* most phases of development take longer and the prepatent period is13 –16 weeks. These parasites are hermatophrodytes, and one adult *F.hepatica* in a bile duct may produce 20 000 eggs per day establishing patent infestation (Robert *et al*., 2010). The life cycle of fasciola maintained by the intermediate host (snail) and final host as it is expressed in the below figure 3.

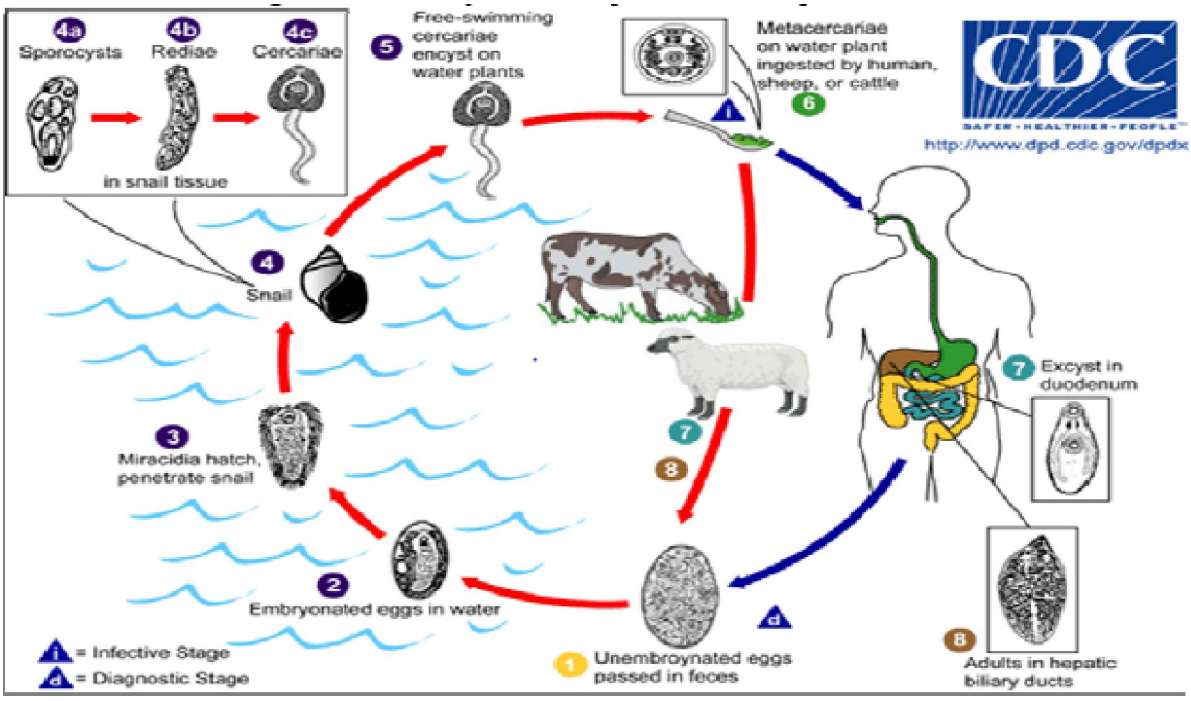

Figure 3: Life cycle of *Fasciola*. Source: (Robert *et al*, 2010).

Pathogenesis

The pathogenesis of fasciolosis varies according to the phase of parasite development in the liver and species of host involved. Essentially the pathogenesis has two phases. The first phase (the parenchyma) (migratory) phase occurs during migration in the liver parenchyma and is associated with liver damage and hemorrhage. *F. hepatica* has a strong predilection for the tissues of the liver. Occasionally, ectopic locations of flukes such as the lungs, diaphragm, intestinal wall, kidneys, and subcutaneous tissue (WHO, 2006). In early infection, during fluke migration, there is hyper proteinemia, hyper globulinemia, and hypo-albuminemia. The hypo-albuminemia is associated with plasma volume expansion caused by liver damage and reduced albumin synthesis. The second phase (biliary phase) occurs when the parasite is in the bile duct which resulted from the hematophagic activity of the adult fluke and damage to the biliary mucosa by their cuticular spines (Yeneneh *et al*., 2012). Hypertrophy of biliary ducts associated with obstruction of the lumen occurs as a result of tissue damage (Tesfaheywet, 2012). Both juveniles and adults feed by secreting enzymes (proteases) which break down blood and liver tissues (Williams *et al*., 2014). Sudden death can occur, in particular in sheep and goats, due to extensive blood loss caused by liver haemorrhage, liver failure. Once an animal is infected with Fasciolosis, Secondary infections, black disease (infectious necrotic hepatitis) obviously occurred: This is an acute and fatal liver disease of ruminants such as sheep, especially, but also cattle. It is caused by a toxin produced by the bacterium *Clostridium novyi*. Tissue destruction by wandering flukes may create a microenvironment favoring activation of Clostridial spores (WHO, 2006) and (Merck, 2016).

Clinical sign

Clinical signs of Fasciolosis are always closely associated with infectious dose (amount of ingested metacercariae). In sheep, as the most common definitive host, clinical presentation is divided into 4 types (Behm and Sangster, 1999).

- **Acute Type I Fasciolosis**: Infectious dose is more than 5000 ingested metacercariae. Sheep suddenly die without any previous clinical signs. Ascites, abdominal hemorrhage, icterus, pallor of membranes, weakness may be observed in sheep.
- **Acute Type II Fasciolosis**: infectious dose is 1000-5,000 ingested metacercariae. As above, sheep die but briefly show pallor, loss of condition and ascites.
- **Subacute Fasciolosis**: infectious dose is 800-1000 ingested metacercariae. Sheep are lethargic, anemic and may die. Weight loss is dominant feature.
- **Chronic Fasciolosis**: infectious dose is 200-800 ingested metacercariae. Asymptomatic or gradual development of bottle jaw and ascites (ventral edema), emaciation, weight loss.

Diagnosis

Diagnosis of Fasciolosis may consist of tentative and confirmatory procedures. A tentative diagnosis of Fasciolosis may be established based on prior knowledge of the epidemiology of the disease in a given environment, observations of clinical signs, information on grazing history and seasonal occurrence. Confirmatory diagnosis, however, is based on demonstration of *Fasciola* eggs through standard examination of feces in the laboratory; postmortem examination of infected animals demonstrates immature and mature flukes in the liver. The latter is helpful in deciding the intensity of infection. There are other laboratory tests (enzymatic and/or serological procedures used to qualify the infection mainly for research purposes. Serological assays are often used to detect infections due to immature forms where fecal egg output is often nil. Such tests allow the detection of substance like Cathepsin L proteases, excretory and secretor products, detection of Ag in milk and ELISA detection of antibodies against the fluke's plasma concentration of Gamma-Glutamyl Transferase (Yadav, 2015).

Treatment

A number of compounds are available for treatment of *Fasciola*; Oxyclozanide (10mg/kg, s/c), Rafoxanide (7.5mg/kg; po), Closanttel (5mg/kg; po), Triclabendazole (10mg/kg; po), Netobimin (20mg/kg; po), Nitroxynil (10mg/; s/c), Albendazole (7.5mg/kg; po) and Clorsolon (7mg/kg; po). In recent year, nitroxynil and clioxandine have been found to be effective against both juvenile and adult flukes (Gebreyohannes and Mekete, 2015). For the treatment of acute ovine Fasciolosis, it is essential to choose a product highly effective against the juveniles that damage the liver parenchyma. For chronic disease a compound active against adult fluke is required. In all drugs higher does must be used against juvenile flukes than against adult in bile ducts. Triclabendazole is considered as the most common drug due to its high efficacy against adult as well juvenile flukes. It is ovicidal and well kills any *F. hepatica* eggs present in the bile duct or the alimentary tract at the time of treatment Albendazole and Rafoxanide affect only the adult stages of *Fasciola* species while the immature stages developed to reach adult stages so that it may be explained why eggs still discharge in this treated group (Radostits *et al.,* 2007).

Prevention and Control

Reduction of Snail Population

Controlling of snail that acts as an intermediate host of *Fasciola* can be done by chemical

control. Although chemical control is effective, snails cannot be eradicated by chemicals because they reproduce so readily. Improved drainage: Irrigation projects can provide habitats to the snails. Cleaning of vegetation regularly may reduce the contamination of herbage (Acici and Bolukbas, 2013).

Anthelmintic therapy

Drugs play a crucial role in the control of Fasciolosis. It is true that seasonal strategic application of effective flukicides which are specific for *Fasciola* as well as timely prophylactic and curative treatment play an important role in the control of liver fluke infection (Wakuma, 2009).

Anthelmintic resistance

Not all compounds are equally effective against stages of development of parasite in the body due to the development of drug resistance. Drug resistance is the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage (Geary et al., 2012). It is a threat to agricultural incomes, and has been reported from all the four corners of the world, to all available drugs, in all classes of helminthes. Drug effects can be evaluated in terms of potency, efficacy, or effectiveness (Lalchhandama, 2010).

Drug efficacy is the maximal effect of the drug that can produce.

Efficacy in pharmacodynamics refers to the capacity of a drug to produce an alteration in a target cell/organ after binding to its.

Potency is a comparative measure, refers to the different doses of two drugs needed to produce the same effect.

Effectiveness: refers "how the drug works in a real world situation, "and is "often lower than efficacy because of interactions with other medications or health condition of the patient, receptor (Adams, 2000). flukicid resistance was not recognized till the report of Triclabendazole resistance in Australia in 1995. And has since been described in Netherlands, UK and Ireland. Although resistance in flukes has not yet reached the levels present in nematodes, resistance exists for the Triclabendazole (khan, 2017) and Albendazole (Desalew, 2014) respectively.

Mechanism of Anthelmintic Resistance

Anthelmintic can arise in a limited number of ways: (1) change in the molecular target, so that the drug no longer recognizes the target and is thus ineffective; a change in metabolism that inactivates or removes the the unselected proportion of the parasite populations drug, or that prevents its activation; (2) a change of distribution of the drug in the target organism that accessing its site of action; or amplification of target genes to overcome drug action (Nicholas, 2004). Benzimidazole (Albendazole, triclabendazole and others) acts by inhibiting of the polymerization of β-tubulin to form microtubules and it is clear that resistance is associated with the point of mutation in β-tubulin genes that prevent drug binding site. However, several different polymorphisms of

genes have been correlated with Benzimidazole resistance (Feng, 2003).

Potential risk factors

As they are expressed in the diagram below drug resistance is affected potentially by different factors.

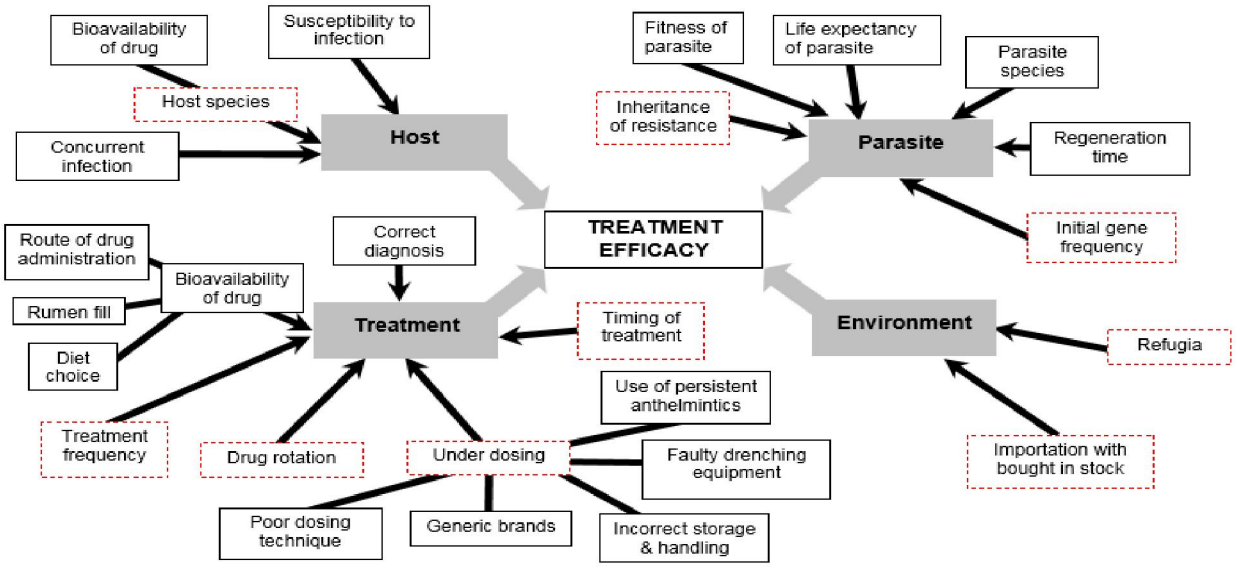

Figure 4: Diagrammatic representations of the potential risk factors of drug resistance in ruminants: Source: (Bartley, 2008)

Methods of anthelmintic resistance detection

Drug susceptibility or resistance from the field and in the laboratory can detected using the In vivo tests (fecal egg count reduction test (FECRT) and controlled efficacy test (CET) also known as "drench and slaughter) and In vitro tests (Egg Hatch Assay, Larval Paralysis and Motility Assay, Larval Development Assay and Tubulin Binding Assay. In vitro tests are majority almost exclusively used for research purposes. These tests can be used to quantify the level of resistance but they require considerable technical expertise and in some cases, expensive laboratory equipment. In vivo tests are the cornerstone of anthelmintic resistance detection in the field. Two tests are commonly used: controlled efficacy test (CET and the fecal egg count reduction test (FECRT). Controlled efficacy test is the test which assesses treatment efficacy in infected animals compared to untreated control animals by estimation of total worm burdens at post mortem (Coles et al., 2006). Fecal egg count reduction test (FECRT) assesses the reduction in fecal egg counts of treated animals expressed as the percentage reduction compared to an untreated control group (Coles et al., 1992). According to the (WAAP, 1995) guide line drug is considered effective when the efficacy is greater than 95% and with >90 lower confidence limit. Unless, resistance is suspected. This is standardized and mainly used for anthelmentic resistance of nematode, but also used for detection of resistance of flukes (Wood, 1995).

Conclusion And Recommendations

Fasciolosis is an economically important parasitic disease of domestic livestock that obstacles production. Effectiveness of treatment measure depends on the drug choice because, not all compounds are equally effective against stages of development of parasite in the body due to the development of drug resistance. Therefore, based on the above conclusion the following recommendations are forwarded;

✓ Effective snail control measure should be conducted.

✓ Treatment should be based up on evaluation of efficacy.

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