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Full Length Research Paper

Study on molecular characterization of rabies virus N gene segment from different animal species in the Sultanate of Oman

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The present study was conducted to determine the molecular characterization of nucleoprotein (N) gene segment of the rabies virus (RV) in Sultanate of Oman. A portion of nucleotide sequence of N gene was amplified from the brains of 4 rabid animals (two goats, one sheep, and one camel) collected during 2011 to 2012, through reverse transcription polymerase chain reaction (RT-PCR). The nucleotide sequences of the gene were subjected to molecular and phylogenic analysis. The phylogenic analysis was performed in relation to Omani isolates and other sequences from the neighboring countries available in the GenBank. Results indicated that one genetic group of virus appeared to be circulating in the Sultanate of Oman with 99% of homology. The study also indicated that the Omani rabies virus is closely related (> 97% homology) to viruses isolated from Saudi Arabia, Jordan and Israel. It is clear from the results that since its first diagnosis in 1990 the rabies virus has not gone through significant variations from the one isolated from first outbreaks. Since, there is no evidence of any other rabies virus variant in Oman, it seem that rabies is maintained endemically in Oman through wild foxes.

Key words: Rabies, Oman, molecular characterization, phylogenetic analysis, Oman.

INTRODUCTION

Rabies is an infectious viral disease of the central nervous system characterized by severe encephalomyelitis, change in behavior, paralysis and death. It is endemic in almost all parts of the world, with the exception of few countries including New Zealand Australia, Hawaii, the United Kingdom and Japan (Seimenis, 2008).

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Globally, rabies is categorized as either urban (where dogs and cats are the major reservoir hosts), and sylvatic (major reservoirs are foxes, wolves, bats and other wildlife) rabies (World Health Organization, 2013). Based upon genetic similarity, there are 7 recognized genotypes of the virus circulating in the world and genotype 1 is responsible for the classical disease throughout world (David et al., 2007). The principal route of rabies transmission is through animal bites however, other routes such as aerosol (Beran and Steele, 1994) ingestion and transplantation have also been documented (Kaplan et al., 1986).

Rabies was first recorded in the Sultanate of Oman in August 1990, when a school boy was bitten by a fox (Anonymous, 1991; Novelli and Malankar, 1991). Within a year, rabies spread was recorded among red foxes (*Vulpes vulpes*) countrywide and human cases occurred in 1991 (wolf bite), 1992 (fox bite) and 1997 (fox bite) (Anonymous, 1998). Unlike other developing countries, dogs are not central to rabies transmission in Oman; foxes are considered the main reservoir (sylvatic transmission) of rabies (Novelli and Malankar, 1991; Ata et al., 1993; Anonymous, 1998; Scrimgeour and Mehta, 2001; Hussain et al., 2013). All the recorded human cases (n = 8) of rabies in Oman have the history of animal bites (mostly fox bite) and the last human case was through a fox bite recorded in 2003 (Anonymous, 2004). The record of Animal Health Research Center (AHRC), responsible for the surveillance and monitoring of rabies in Oman indicated the disease is still prevalent among different species of livestock.

Rabies virus belong to the genus Lyssavirus in the Family Rhabdoviridae and consist of non-segmented negative single-stranded RNA genome that encodes five structural proteins, nucleoprotein (N), phosphoprotein, matrix protein, glycoprotein (G) and RNA-dependent RNA polymerase (Wunnwr et al., 1988; David et al., 2001). Recent advances in technology have contributed to better understanding of molecular epidemiology and geographic relationships of rabies virus isolates (Susetya et al., 2008; Zhang et al., 2009). The nucleoprotein (N) gene has been extensively used for genetic typing and evolutionary studies because of its relatively conserved variation among reservoir-associated variants and geographic lineages (Bourhy et al., 1993; Wiktore tal., 1980). Moreover, the nucleotide sequence of the N gene used extensively as a molecular marker to explain the patterns of the geographic distribution of rabies virus at the regional and global level (David et al., 2000). There is no information available on the relationship of rabies virus variants to each other or to the host species in Oman, as also, limited information is available on link between these variants in Oman and the submissions from neighboring countries (David et al., 2000, 2007). Therefore, determining the genotype of the circulating rabies virus was important to elucidate the dynamic of disease transmission. Keeping in view the afore mentioned scenario, current study was conducted to understand the molecular characterization of rabies in the sultanate of Oman by sequencing the N gene fragment of virus obtained from the samples submitted for the routine diagnosis to AHRC.

MATERIALS AND METHODS

Virus detection

Either carcasses from different rabies suspected animals species or their chilled/frozen heads (n = 257) were submitted to the virology section of AHRC from all over Oman during 2011 and 2012. Rabies was confirmed in 135 (52.5%) of these animals by a commercial direct immunofluorescent test (FAT) using Anti-Rabies Monoclonal Globulin (Fujirebio Diagnostics Inc. 201 Great Valley Parkway Malvern, PA 19355, USA) (Wiktor and Koprowski, 1978; Wiktor et al., 1980).

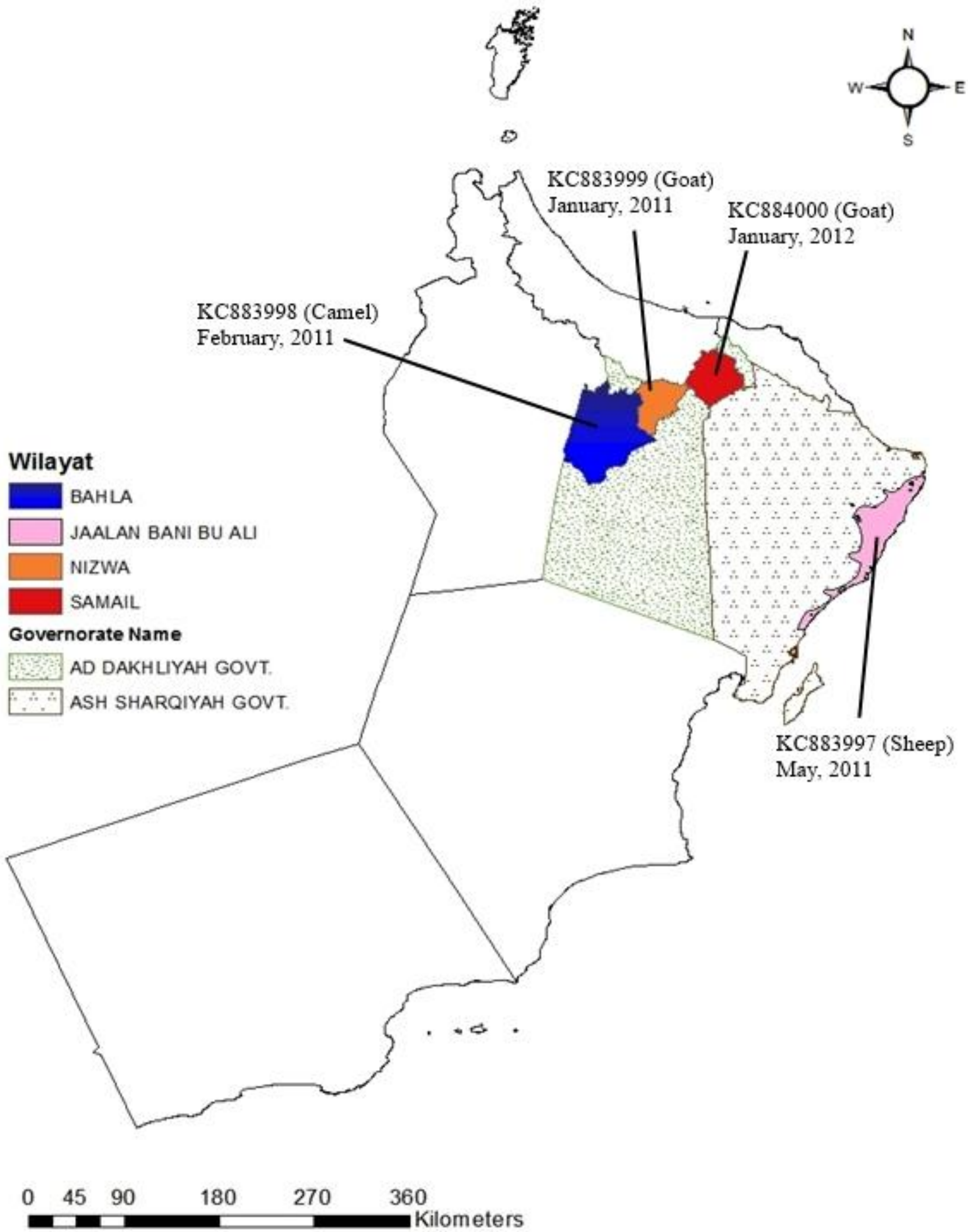
Ten of the FAT positive samples were used for the RT-PCR analysis in this study and 4 samples out of these were further subjected to sequencing. The species of origin location of sequenced samples and the year are shown in Map 1.

Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 0.2 to 0.5 g of brain tissue using QIA amp Viral RNA kit (QIAGEN, Germany) following the manufacturer's protocol. The RNA was dissolved in the RNase free buffer provided with kit. PCR was performed using one step RT-PCR kit following the manufacturer's protocol. For the amplification, the N gene specific primers were designed to target 355 bp conserved regions based upon the previous submissions in the GenBank (Singh et al., 2010). Briefly, 25 ul reaction mix was prepared by using 5 ul of purified RNA template, 5ul of 5× QIAGEN one-step RT-PCR Buffer, 3ul of 5× QIAGEN Q solution, 1.5 of dNTP Mix (10 mM), 3 ul (30 pmol) each of N gene specific forward primer RabN-533F;5-CATTGCAGATAGGATAGAGC-3 and Reverse primer; Rab N-888R;5-GAGGAACGGCGGTCTCCTG-3, 1 ul of QIAGEN one-step RT-PCR enzyme mix, 0.5 ul of RNase inhibitor (20 U/ul), and 3 ul of H_2O. The reaction was incubated at 60°C for 1 min, 42°C for 10 min, and 50°C for 30 min for cDNA synthesis (step 1) followed by denaturation at 95°C for 15 min (step 2). After initial denaturation, the amplification was carried out for 30 cycles of three steps, at 95°C for 30 s, 55°C for 30 s, 75°C for 45 s (step 3), with final extension at 72°C for 5 min.

Sequencing of N gene nucleotide

PCR fragments were sequenced using the ABI PRISM BigDye terminator cycle sequencing kit (Perkin Elmer, USA) with primers RabN-533F and RabN-888R, and run on an ABI 310 genetic analyzer. The phylogenetic tree was constructed with neighbor-joining method (Saitou and Nei, 1987) in MEGA (Molecular Evolutionary Genetic Analysis) software (Kumar et al., 2001). Phylogenetic analysis was carried out for 355 nucleotides of each sequence. The sequences of 100 rabies viral isolates submitted from neighboring countries and world were retrieved from GenBank and used for phylogenetic survey. The dendrogram branch lengths represented the predicted number of substitutions. The nucleotide



Map 1. Map of Sultanate of Oman showing the geographical location of the rabies positive animals used in this investigation.

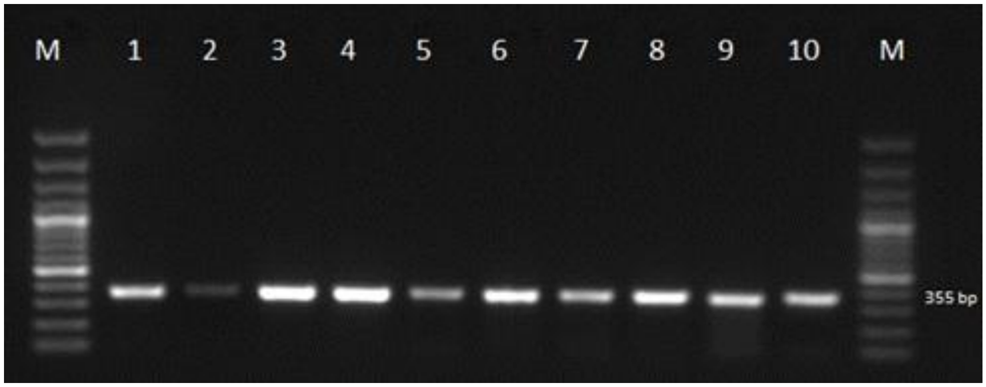


Figure 1. Agarose gel electrophoresis (1.5%) of RT-PCR products generated from rabies brain samples (n=10) collected from different FAT positive animals. Four samples (Lane 3: Goat, Lane 5: Sheep, Lane 6: Goat, Lane 9: Camel) were used for the sequencing.

sequences described in this report were submitted to GenBank and assigned the following accession numbers: KC883997, KC883998, KC883999 and KC884000.

RESULTS

During the study period, 257 suspected samples were submitted to AHRC during 2011 to 2012. Off these, 135 samples were found positive for rabies through FAT. Ten of FAT positive cases were subjected to RT-PCR using N gene specific primer test. A specific product of 355 pb was detected in brain samples of different species on agarose gel electrophoresis (Figure 1).

Similarity and phylogenetic analysis

Out of the 10 RT-PCR positive samples, four samples (sheep (1), goat (2) and camel (1) origin submitted from Al Dakhiliyah and Ash Sharqiyah governorates of Oman were sequenced and submitted to GenBank. The details of samples are given in map 1. A total of 355 bp nucleotide sequence encoding the nucleoprotein from 4 FAT and RT-PCR positive samples was determined. The N gene sequences of other rabies isolates obtained from GenBank database were compared with those of 4 Omani isolates to study the phylogenetic relationship. Phylogenetic characterization of the sequenced fragments revealed that the four submissions grouped into one cluster, together with other Omani. The nucleotide similarity among 4 Omani isolates was 99% and with other Omani isolates was found to range from 99- to 98%. Moreover, the Omani isolates collected from foxes during first outbreak (1990s and 1991) were submitted to GenBank in 2000 (EU086166) and 2010 (GU992306) by Pasture Institute also had 98% homology with analyzed samples. Based on these findings red foxes were found to play an important role in the epidemiology of rabies in Sultanate of Oman since 1990. The Omani samples were found genetically indistinguishable from those obtained from foxes from other countries in Middle East and the submissions in this study has 98% homology with the viruses isolated from foxes in Saudi Arabia (U22480 in 1987, foxes from Israel (DQ37450; EU08613) in 2000 and 98 to 97% homology with the viruses isolated during 1998 and 1999 from Jordon (Figure 2), suggesting that only sylvatic rabies virus is circulating in Oman and foxes acted as an important reservoir host in transmitting rabies to domesticated animals and humans in this region.

DISCUSSION

The current study was conducted to characterize Omani rabies virus N gene segments by using molecular and phylogenetic analysis. PCR and sequencing assay described in this study greatly enhanced our ability to make a sensitive and specific diagnosis as well as typing of animal viruses, including rabies virus, which is an important tool that provides a better understanding of epidemiological relationships (Kissi et al., 1995; Haas, 1997). The nucleotide sequence analysis of the 355 bp fragment of the N gene of isolates represented the first molecular epidemiological study of rabies virus from Oman.

The highly conserved nucleoprotein (N) gene of rabies virus is the most popular target for the diagnosis by using reverse transcription polymerase reaction (PCR) (Ito et al., 1999; Arai et al., 2001). However, it also allows viral strains to be differentiated accurately by analyzing genetic differences present within the gene (Johnson et

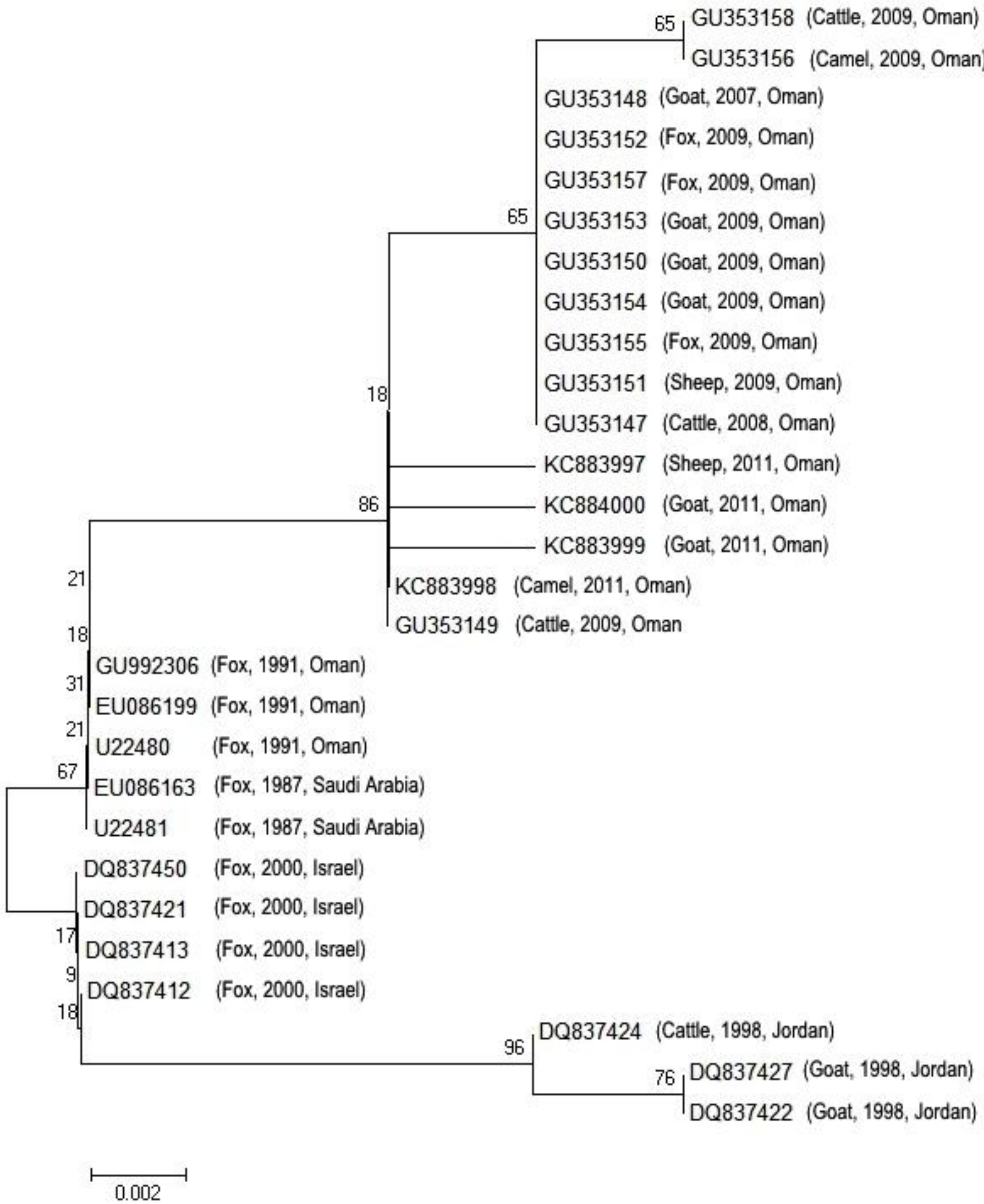


Figure 2. Phylogenetic tree based on N gene nucleotide sequences of the Omani rabies isolates sequences from Saudi Arabia, Israel and Jordan

al., 2002). The molecular sequence and phylogenetic analysis of Omani rabies virus isolates described here, has demonstrated a high degree of similarity between isolates originating from different species which is in agreement with previous finding that rabies virus isolates from different species usually clustered according to geography and not according to the species (Nagarajan et al., 2006; David et al., 2007).

Identification of host population critical to the maintenance of rabies in a region, as opposed to populations in which outbreaks are the result of 'spillover' infections is of uttermost importance in devising the appropriately directed control measures. Our finding further strengthened the claim that rabies virus has established a sustained transmission network in foxes (reservoir host) in Oman (Novelli and Malankar, 1991; Hussain et al., 2013). Foxes were previously indicated as important wildlife reservoir host in Middle East and world (Seimenis, 2008). The close relationship among these isolates and evolutionary clustering of rabies virus isolates from 3 species (sheep, goat and camel) with samples submitted from foxes affected back in 1991 in Oman (GU992306, EU086199 and U22480) and their different geographical regions of collection (Map 1) in this study suggested that virus clustering was not due to geographical origin or host species in Oman.

The phylogenetic analysis indicated that samples originating from Oman were closely related (> 97% homology) to viruses isolated from Saudi Arabia, Jordan and Israel. The isolates from these countries were found closely related in previous studies as well (David et al., 2000, 2007) and well supported by the established paradigm of rabies epidemiology that throughout the world rabies virus had evolved into distinct variants that tend to establish in a region with relatively low species density (Lembo et al., 2007).

Conclusion

Our results indicated that based on phylogenetic analysis Omani rabies virus submissions are closely related and the foxes play an important role as a reservoir host of rabies in Oman. Their population and movement could be responsible for the distribution of virus in the country. Application of bait vaccine for wild animals could be used around the place of outbreaks to control the disease in affected areas. The genetic surveillance of circulating rabies virus could become an important tool in monitoring and further understanding the epidemiology of rabies in the Sultanate of Oman.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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Review

Rational veterinary drug use: Its significance in public health

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Veterinary drugs are used as therapeutic, prophylactic and growth promotion, and can be used in either rational or irrational way. Rational use of drugs means the sick animals receive medications appropriate to their clinical needs, in doses that meet their own individual requirements, for an adequate period of time, and at the lowest cost to them and their community. Irrational drug uses are characterized by over-prescription, inappropriate dosage, incorrect duration and unnecessary risk. There are several reasons which may contribute to irrational use of drugs. Some of them are lack of information, inadequate training and education of graduates of veterinary medicine, poor communication between veterinarian and owner, lack of diagnostic facilities, demand from the animal owner, and promotional activities of pharmaceutical industries. Hence, the potential public health hazard as a result of irrational drug use in food animals includes limited efficacy, increase risk of unwanted effects such as the emergence of drug resistance and drug residue, waste of resources and psychosocial impacts. However; rational use of drugs in veterinary medicine has manifold significance; it can be either public health significance, improve food safety concern, reduce the development of drug resistance and residue or economic significance; the need to rely on more expensive drugs and international trade barrier. Some of the measures that promote rational drug use are: herd health management, alternatives for antimicrobials growth promoters, adhering to withdrawal period and minimizing misuse of antimicrobials.

Key words: Rational/irrational use, veterinary drugs, drug residue, and resistance

INTRODUCTION

Drugs in animals can be used for various purposes, such as therapeutic, prophylactic (Hirsh and Zee, 1999), growth promotion and other uses (Kanneene and Miller, 1997). When veterinary drugs are indicated rationally in right dose and route of administration, the potential damages of their use are reduced and their

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efficacy increased (Vitomir et al., 2011). Rational use of drugs in veterinary medicine has both public health (FAO/OIE/WHO, 2003) and economic significances (World Health Organization(WHO), 2001).Non-rational use of drugs in veterinary medicine, as well as the need for control of their use becomes even bigger problem when used on food producing animals (Vitomir et al., 2011). The irrational antimicrobials use causes a particular concern for unwanted residues in foods of animal origin and for the development of antimicrobial resistance (Catery et al., 2003). To maintain the efficacy of antibiotics and use them for long lasting and to reduce the risk of resistant bacteria development, the use of antibiotics must be restricted (WHO, 2001).

Maintaining all food animals in a clean healthy environment and developing a nutritional program to meet growth, maintenance and lactation needs of animals are crucial. Hence, drug misuse is best avoided by implementing management practices and health programs that keeps animals healthy and producing efficiently (American Veterinary Medical Association (AVMA) and National Milk Producers Federation (NMPF), 1999; Radostitis, 1994). Therefore, this review provides an overview on importance of rational veterinary drug use in food safety, its public health and economic significances, and the control measures of irrational veterinary drug use in food of animal origin.

USES OF VETERINARY DRUGS

Drugs in animals can be used as in therapeutic, prophylactic and growth promotion. Therapeutic use refers to the treatment of established infections whereas prophylaxis is the use of drugs in either individual or groups to prevent the development of infections. Both therapeutic and prophylactic uses involve administrations of drugs by different routes at therapeutic levels for short period of time (Hirsh and Zee, 1999). The use of antimicrobials as feed supplements can promote the growth of food animals and also enhance feed efficiency (Graham et al., 2007; NAS, 1999; Kanneene and Miller, 1997).

Therapeutic and prophylactic

Most drugs, around 60%, are used for therapeutic purposes in humans, although an increasing amount is administered as prophylaxis to prevent infection, the farming industry is the second largest consumers of antimicrobials after medical practitioner. Depending on action, prophylactic and therapeutic drugs may be divided into different groups. The most widely distributed drugs are antimicrobials, antiparasitic and antimycotic preparations and their use in prevention and treatment of animal diseases (WHO, 2001). Veterinary drug products licensed for use throughout the world do not vary greatly from country to country, although the level of use, withdrawal times accepted safe level in food do change from country to country in almost all cases. Depending on the requirements in different countries, feedstuffs containing veterinary drugs may be available only on the prescription of a veterinarian or they may be freely available. In most circumstances, if recommended withdrawal periods are observed, the presence of unacceptable residues is not expected (Fingleton, 2004).

The choice of antimicrobial drugs to use for the treatment of animal diseases caused by an infectious agent should be in line with the guidelines as it aids in decision making process. The gold standard for this determination is the result of microbiological culture. However, strict application of this standard is unrealistic because the decision to use antimicrobial drugs is made several days before culture data are available. Therefore, as an aid in determining that a particular process has infectious component, certain clues are used. The Infectious Control Committee at Veterinary Medical Teaching Hospital, University of California has drawn up guidelines for rational use of antimicrobial drugs which are: demonstrations of an infectious agent; clinical data (at least two of the following): fever; leukocytosis; localized inflammation and radiographic evidence. The whole purpose of the exercise is to know whether there is an infectious agent present or the best antimicrobial drugs to be used (Hirsh and Zee, 1999).

Growth promoters (GPs)

“Growth promoters’’ are any antimicrobial agents administered at low or subtherapeutic dose and destroys or inhibits growth of microbes as infectious agents reduce the yields of food animals (Peter and John, 2001). Antibiotic GPs are used to help growing animals by increasing the rate of weight gain and to improve feed conversion efficiency (Graham et al., 2007; Jensen, 1998), and get maximum benefit from it and allow them to develop into strong and healthy individual.

Although the mechanism underlining their action is unclear, it is believed that the antibiotic suppresses sensitive populations of bacteria in the intestines. It has been estimated that as much as 6% of the net energy in the pig diet could be lost due to microbial fermentation in the intestine. If the microbial population could be controlled, it is possible that the lost energy could be diverted to growth (Jensen, 1998). It is also hypothesized that cytokines released during immune response as a result of bacterial infection may stimulate the response of catabolic hormones, which could reduce muscle mass. Therefore, a reduction in gastro intestinal tract (GIT) infections would result in the subsequent increase in muscle weight.

Whatever the mechanism of action, the result of the use of GPs are resulting in meat of better quality, with less fat and increased protein content (Peter and John, 2001). There can be no doubt that GPs are effective; however, the effects of GPs are more noticeable in sick animals and those housed and confined, and unhygienic conditions (Prescott and Baggot, 1993). The use of GP is largely a problem of intensive farming methods and the problems caused by their use are largely those of developed rather than developing countries (Peter and John, 2001).

OTHER USES OF VETERINARY DRUGS

Preservation and processing of food

In preservation and processing, food additives are added to prevent the onset of spoilage, to promote the binding properties and to enhance flavor and nutritive value. These additives include antioxidants, sequestrants, coloring agents, stabilizers, sweeteners, tenderizers, etc. At both production and processing stages, residues or contaminants may enter the food chain from intentional exposure to these chemicals (Gracey et al., 1999).

Pre slaughter control of stress in abattoir

Certain neuroleptic drugs (tranquilizers) are administered to avoid excitement of animal or to curb aggressive behavior. The misuse of such drugs and some beta-adrenergic blocking are used to reduce the stress of transportations to the slaughter house raises concern from the view point of consumer protection. Residues of drugs given for these purpose will remain at a high level in edible tissues, since animals are slaughtered shortly after the drug is administered and while the concentration of the drug remains at therapeutically effective levels (FAO, 1984).

Control of reproduction

Prostaglandins and their analogs and sex steroids are used to regulate fertility and breeding programmes. Glucocorticoids and prostaglandins are used as abortifacients or to control timing of parturition. Animals would not be slaughtered shortly after the treatment and residue problem in meat could only occur in the event of causality meat slaughter (FAO, 1984).

WAYS OF VETERINARY DRUG USE

Rational veterinary drug use

Rational use of medicines in veterinary science is defined as that sick animal should receive medications appropriate to their clinical needs, in doses that meet their own individual requirements, for an adequate period of time, and at the lowest cost to them (WHO, 2002). These requirements will be fulfilled if the process of prescribing is appropriately followed. This includes: steps in defining patients' problems (or diagnosis); in defining effective and safe treatments (drugs and non-drugs); in selecting appropriate drugs, dosage and duration; in writing a prescription; in giving patients adequate information; in planning to evaluate treatment responses (WHO, 1988).

Irrational veterinary drug use

Irrational drug use or inappropriate drug uses are characterized by over-prescription (prescribing drug when none are needed clinically), omission (when required drugs for conditions are not prescribed), the use of inappropriate dosage (too high or too low), incorrect duration (too short or too long), incorrect selection (mismatch between organism) and unnecessary risk (use of injection or intravenous antibiotic when oral forms would be suitable) (Brahma et al., 2012). The irrational drug use causes a particular concern for the development of resistance. Antimicrobial drug is now becoming a major problem both in veterinary and human medicine as consequence of the intensive use and misuse of antimicrobial drugs (Catery et al., 2003). There is a wide spread misuse of permitted drugs which result in unwanted residues in foods of animal origin. Also, there has been wide spread failure to observe the recommended withdrawal and withholding period for antimicrobial agents (Thawani, 2010).

REASON FOR IRRATIONAL USE OF DRUGS

There are several reasons which may contribute to irrational use of drugs. These are: lack of information; faulty and inadequate training and education of medica and/or veterinary graduates; poor communication between health professional and animal owner; lack of diagnostic facilities/uncertainty of diagnosis; demand from the owner (to satisfy the patient expectations and demand of quick relief, clinicians prescribe drugs for every single complaint). Also, there is a belief that "every ill has a pill". All these increase the tendency of poly-pharmacy, defective drug supply system and ineffective drug regulation (absence of well-organized drug regulatory authority and presence of large numbers of drugs in the market leads to irrational use of drugs) and promotional activities of pharmaceutical industries (the lucrative promotional programmes of the various pharmaceutical industries influence the drug prescribing) (Brahma et al., 2012; Shivhare et al., 2010).

IMPACTS OF IRRATIONAL USE OF DRUGS

Irrational use of drugs can have a negative impact on the public health, some of them are as follows: reduction in the quality of drug therapy (limited efficacy) leading to increased morbidity and mortality; increase risk of unwanted effects such as adverse drug reactions and the emergence of drug resistance due to widespread overuse of antibiotics as well as their use in under-therapeutic dosage; waste of resources leads to reduced availability of other vital drugs and increased costs and adverse, possibly lethal effects, for example due to antibiotic misuse or inappropriate use of drugs in self-medication (Brahma et al., 2012); psychosocial impact, such as when patients come to believe that there is "a pill for every ill", which may cause an apparent increased demand for drugs (Grandle et al., 1993).

SIGNIFICANCE OF RATIONAL VETERINARY DRUG USE

Rational use of drugs in veterinary medicine has public health and economic significances.

Public health significance

No significant reported episodes of adverse human health effects occurring in food when the veterinary drugs were used at the correct dosages and at the levels permitted (FAO/OIE/WHO, 2003).

Improve food safety concern

When drugs indicated rationally, the potential adverse effects of their use as a result of consumption of animal products. However, in non-rational use of drugs in veterinary medicine, mainly when used on food producing animals, there is the possibility that minimal quantities of drugs and their metabolites (residues) which remain in animal products (meat, milk, eggs and honey) and induce certain harmful effects in people as potential consumers of such food (Vitomir et al., 2011).

Reduce the development drug resistance

Human health can be affected by a widespread of antibiotic resistance pathogens, as it is occurring due to extensive overuse of antibiotics, as well as their use in under-therapeutic dosage (Brahma et al., 2012). Resistant microorganism can get access to human either through direct contact or indirectly via meat, milk, egg. As the bacteria, the endogenous flora of food animal, contaminate food of animal origin, might either colonize human or transfer resistant gene to humans endogenous flora or super impose an additional load to the reservoir of resistant genes already present in man (Stobberingh and Bogaard, 2000).

The use of antibiotics in food animal can result in antibiotic resistance bacteria reaching the human population through variety of routes. Antimicrobial resistant bacteria such as *Escherichia coli* can colonize intestines of heavily exposed humans (farmers, who used food containing antibiotics, slaughter house workers, cookers and other food handlers) often have a higher in-cidence of resistant *E.coli* in their feces than the general population. Contaminated meat by intestinal bacteria at slaughter is extensive and an important route by which resistant bacteria reach people. While many bacteria are non-pathogenic, some pathogenic bacterial species from the intestines of animals causes zoonotic infection to humans such as *Salmonella* species, *Campylobacter jejuni* and these infection may be harder to treat because it is acquired by humans and are a potential source of resistance plasmids for human pathogenic bacteria other than zoonotic infection (Hirsh and Zee, 1999). Rational use of drugs can also signifi-cantly minimize the risk of microorganisms resistance development (in case of antimicrobials). Hence, no significant reported episodes of adverse human health effects occurring in food when the veterinary drugs were used at the correct dosages and at the levels (FAO/OIE/WHO, 2003).

Reduce the development of drug residue

Veterinary drug residues are one of the major problems for food contamination. Human health can be affected through residues of drugs in food of animal origin, which may cause direct side effects. In general, the effect of antibiotic residue in food of animal origin is significant when compared with the antibiotic misuse or selection and amplification of antibiotics resistant strain of bacteria (Peter and John, 2001).

Food animal origin such as meat, milk and eggs intended for human consumption, may have some residual amounts of veterinary drugs which remains in edible tissues after harvest. In some countries where legislative directions are followed by the farmer/producer, drug residue level will be within safe limits. In a relatively few cases, however, levels of residue exceed permitted maximum limits. This is attributed to the improper/ irrational drug use and as such, it is not legally allowed into the food system (WHO, 2000). Generally, there is no significant reported episodes of adverse human health effects occurring in food when the veterinary drugs were used at the correct dosages and at the levels permitted (FAO/OIE/WHO, 2003).

Economic significance

A wide spread availability and use of antimicrobials have several negative implications on global health care: among these developments of drug resistance is one. The primary economic implications of resistance on the diminishing efficacy of antibiotic treatment includes the need to rely on more expensive drugs that may be practically unaffordable for most primary health care programs (WHO, 2001). Antimicrobial residue remains very significant from the prospective of international trade and consumer confidence, because it results in international trade barrier. As tariffs are removed and goods flow freely between countries, importing countries must be in confident that goods available for purchase are safe, and in addition to this, from time to time, there is pressure to use antimicrobial residues on non-tariffs barrier to importation (Kanneene and Miller, 1997). Major economic loses and animal welfare problems could arise in veterinary medicine, because antimicrobial resistance has been found to cause therapy failure and higher mortality and morbidity rate (Acar, 1997; Kessar, 1997).

MEASURES TO PROMOTE RATIONAL VETERINARY DRUG USE

To avoid entering a post antibiotic era, agents around the world are determining the use and abuse of antimicrobials (WHO, 2001). To reduce the risk of selecting resistant bacteria, the use of antibiotics must be restricted. Thus, the most attractive area for reducing the use of antibiotics is to ban their use as growth promoters. Some of the measures that reduce irrational (promote rational) drug use are as follows:

Herd health management

All food animals should be maintained in a clean healthy environment whenever possible. A nutritional program should in effect meet growth, maintenance and lactation needs. The veterinarian should implement a health program that encompasses preventive medical procedures. Drug misuse or irrationality are best avoided by implementing management practices and health programs that keeps animals healthy and producing efficiently (AVMA and NMPF, 1999; Radostitis, 1994).

Alternatives for antimicrobials growth promoters

Essentially, there are many ways by which we can reduce our dependence on antibiotic use in animals. Developing an alternative to antibiotics that work via similar mechanisms, promoting growth whilst enhancing the feed conversion efficiency, is the best option. A more difficult route would be to improve animal health.

Growth promoters have been shown to perform best when the condition is worst, that is when animal is in poor health and the living conditions are unhygienic; if their local environment is improved with overcrowded reduced and injection control technic is introduced, then the actual need for growth promoter may be removed (Prescott and Baggot, 1993).

Competitive exclusion products

These are in feed microbes consisting of a variety of species of bacteria that are marketed as being “friendly’’. The mechanism of action is believed to be that, by allowing bacteria to colonize the GIT, potential pathogens are prevented from colonizing the gut and thus causing infection. These products are often administered to new born animals, especially poultry, to colonize the GIT and prevent *Salmonella* and *Campylobacter* infections. It is not known how the treatment is but it is believed to reduce diarrhea and level of mortality. These products are also given to animals that have been treated with therapeutic antibiotics, to recolonize a gut that may be depopulated by antimicrobial action of the drug (Peter and John, 2001).

PROBIOTICS

Probiotics are a term used for products containing “beneficial’’ microorganism. Most contain either *Lactobacillus* species (primarily *L. acidophilus*) or *Streptococcus faccium*. Additionally, they may contain vitamins, trace minerals and various growth factors (Haward, 1993). Probiotics are similar to exclusion products.

They are believed to improve the overall health of an animal by improving the microbial balance in its gut. It has been hypothesized that their action can be summarized in three ways. The first is reiteration of competitive exclusion principle by colonizing the gut in large number; the probiotic bacteria exclude pathogens and thus prevent them from causing infection. The second possibility is that they act as a stimulus for the immune system. As the immune system is engaged following exposure to probiotic bacteria; any hostile bacteria are also noticed. Following increased surveillance by leukocytes and thus potential pathogens are eliminated. The third suggestion proposes that probiotics have strong, positive influence on intestinal metabolic activities, such as increased production of vitamin B_{12}, bacteriocins and propionic acid (Peter and John, 2001).

PREBIOTICS

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). In other words, prebiotics are meant to provide a substrate for beneficial GIT microbes. Large amounts of bacteria present in the mono-gastric small intestine and are potentially capable of utilizing these indigestible carbohydrate sources for energy. Some researches (Hillman, 2001; Houdijk et al., 1997) have been conducted to manipulate beneficial bacteria in GIT. The use of prebiotics is promising approach for enhancing the role of endogenous beneficial organisms in the gut as suggested by Bezkorovainy (2001). They can be used as potential alternatives to growth promoting antibiotics (Hatemink, 1995). The use of prebiotics or fermentable sugars instead of antibiotics is going to be popular in birds in order to improve the useful microbial population of the GIT (Kermanshahi and Rostami, 2006).

IN FEED ENZYMES

Enzymes are routinely added to animal feeds and work by helping to break down certain components of the feed that the animal may have problems in digesting. They are produced by fermentation processes from fungi and bacteria and seen to only have a positive effect on the animal. The scientific committee for animal nutrition concludes that conditions of use evaluated so far are acceptable as respected to consumers, users and animals (Peter and John, 2001).

INFECTIOUS CONTROL MECHANISM

The use of antimicrobial as growth promoting agent rests on their role in controlling infection in growing animals. Similarly, many of the alternatives are aimed at controlling infection, often indirectly. For instance, the Australian pig farming pioneered the ‘’all in-all out’’ method of pig production. This is a new system used to replace the older technique of having constant system of pig moving through the farm. Instead of having a range of ages, all the pigs weaned within a week are designated into a single cohort and are housed together in one shed. They are not allowed to mix with pigs from other cohort and so cross infection between groups are prevented. The “Specific Pathogen Free’’ system is used to prevent pigs from acquiring many of the disease that require antibiotic intervention. To achieve this they are born by hysterectomy and hand reared. This will only be cost effective for valuable breeding stock. Finally, vaccination is used to offer protection against certain pathogens, such as enterotoxigenic *E.coli* and various *Mycoplasma* infections (Peter and John, 2001).

ADHERING TO WITHDRAWAL PERIOD

The withdrawal time (also known as the depletion or clearance period) is the time for the residue of toxicological concern to reach safe concentration as defined by the tolerance. Depending on the drug product, dosage form, and route of administration, the withdrawal time may vary from a few hours to several days or weeks. It is the interval necessary between the last administration to the animals of the drug under normal condition of use and the time when treated animal can be slaughtered or the production of safe foodstuffs (Kanneene and Miller, 1997). To ensure that drug residues have declined to a safe concentration following the use of drugs in animals, a specified period of drug withdrawal must be observed prior to providing any products for human consumption. Drug withdrawal time is the time required for drug residues to reach a safe concentration for human or animal consumption, and defined as maximum residual limit (MRL). Failure to follow recommended withdrawal time is often implicated in residual problems (GOV.UK, 2013). It is advisable to follow recommended withdrawal time to avoid residual effects of drugs in the food of animal origin; that is, we have to check and observe the withdrawal period laid down for the particular medicine and food animals should not be sold for slaughter, or slaughtered before the end of withdrawal period (Gracey, 1999).

MINIMIZING MISUSE OF ANTIMICROBIAL

This is achieved by different strategies. These are: educations of prescribers and dispensers (including drug sellers); education of the farmers to create awareness; limiting the availability of antimicrobials to prescription; ensuring that only antimicrobials meeting international standards of quality, safety and efficiency are granted marketing authorization; establishing and maintaining updated national standard treatment guidelines; developing guidelines for veterinarians to reduce overuse and misuse of antimicrobial in food animals and enhancing immunization coverage and other preventive measures, thereby reducing the need for antimicrobial (Gracey, 1999). WHO came out with twelve core interventions to promote more rational use of medicines. Some of them are: public education about medicines; clinical guidelines; appropriate and enforced regulations; supervision, audit and feedback; independent information on medicines and problem based pharmacotherapy training in undergraduate curriculum (WHO, 2004).

CONCLUSION

Although veterinary drugs have played a great role in control and prevention of disease in animals, and promote the growth of food animals , its use is associated with problems such as development of resistance and residual effects in food animals. These adverse effects are generally due to irrational use of drugs such as misuse, extensive use, failure to keep strict adherence of withdrawal and withholding time of drugs. The development of resistant micro-organisms in animal and the presence of drug residue in food of animal origin have significant effect on public health.

Therefore, strict control measures to promote rational veterinary drug use have crucial importance on global enconomy and public health.

ABBREVIATIONS

E. coli, *Escherichia coli*; **FAO,** Food and Agriculture Organization; **GIT,** gastro-intestinal tract; **GP,** growth promoter; **IDU,** irrational drug use; **JECFA,** Joint **FAO/WHO** Expert Committee on Food Additives; **MRL,** maximum residue level; NAS, National Academic Sciences; **OIE,** Office International des Epizooties; **RDU,** Rational drug Use; **Vet,** veterinary; **Vet. Med,** veterinary medicine; **WHO,** World Health Organization; **WP,** withdrawal period.

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Full Length Research Paper

An Abattoir Survey on the Prevalence and Monetary Loss of Fasciolosis Among Cattle Slaughtered at Dangila Municipal Abattoir, Ethiopia

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Bovine fasciolosis is an economically important parasitic disease of cattle in tropical and subtropical countries responsible for considerable economic losses in the cattle industry, mainly through condemnation of fluke-infected liver. A cross sectional study was conducted between October, 2013 and March, 2014 to determine the prevalence and monetary losses associated with bovine fasciolosis and comparing coprology and post mortem techniques of examination among cattle slaughtered at Dangila municipal abattoir in Amhara region. Out of a total of 384 cattle examined, 85 (22.14%) and 116 (30.21%) were positive for fasciolosis through coprological and postmortem examinations, respectively. *Fasciola hepatica* was found to be the predominant *Fasciola* species in the study area with prevalence of 42.2% whereas *Fasciola gigantica*, mixed, and immature or unidentified forms of *Fasciola* species were found to be 27.6, 19.0 and 11.2%, respectively. However, there was no statistically significant variation (P > 0.05) in the prevalence of fasciolosis based on age of the animal, young (< 5 years) were counted more prevalence of 23.1 and 32.7% in coprology and post mortem examinations, respectively than adult (> 5 years) with prevalence of 21.8% and 29.3% in coprological and post mortem examinations, respectively. Statistically significant variation (P < 0.05) was observed in the prevalence of fasciolosis among animals with different body conditions and months both in coprology and post mortem examinations. The highest prevalence of bovine fasiolosis was recorded in poor body conditions, with prevalences of 37.2 and 46.5% in coprology and post mortem examinations, respectively, while the highest prevalence was found to be in October with 30.7 and 40.6% both in coprology and post mortem examinations, respectively. The total monetary loss incurred due to condemned liver and carcass weight loss was estimated to be 945,270 Ethiopian birr (48,432 USD) per annum, indicating the great impact on the economy. Therefore, more detailed studies on ecology and biology of the snail intermediate host and its effective control measures should be planned.

Key words: Abattoir, cattle, Dangilla, Ethiopia, fasciolosis, monetary loss, prevalence.

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa, of roughly 53.99 million animals. Out of this

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total cattle population, the female cattle constitute about 55.48% and the remaining 44.52% are male cattle (Community-supported agriculture (CSA), 2012).

However, this great potential is not properly exploited as a result of diseases, malnutrition and other management problems. Parasitism is one of the major bottlenecks to livestock development in the tropics (Gupta and Singla, 2013). Among many parasitic problems of animals, fasciolosis, caused by trematodes of the genus, *Fasciola,* is a major disease, which imposes direct and indirect economic impact on livestock production, particularly of sheep and cattle. Apart from its veterinary and economic importance throughout the world, fasciolosis has recently been shown to be a re-emerging and widespread zoonosis affecting many people (Kassai, 1999; Chhabra and Singla, 2009).

Fasciola hepatica and *Fasciola gigantica* are the two liver flukes most commonly reported to cause fasciolosis in ruminants. *F. hepatica* has a cosmopolitan distribution, mainly in temperate zones, while *F. gigantica* is found in tropical regions of Africa and Asia (Mihreteab et al., 2010). In Ethiopia, *F. hepatica* and *F. gigantica* infections occur in areas above 1800 meters above sea level (m.a.s.l.) and below 1200 m.a.s.l, respectively which has been attributed to variations in the climatic and ecological conditions such as rainfall, altitude, temperature and livestock management system (Yilma and Malone, 1998).

The life cycle of liver flukes involves a freshwater snail as an intermediate host. The geographical distribution of trematode species is dependent on the distribution of suitable species of snails. The *Galba/ Lymnaea* complex in general and *Galba (Lymnaea)* truncatula and *Lymnaea (Radix) natalensis* in particular are the most common intermediate hosts for *F. hepatica* and *F. gigantica,* respectively. The presence of fasciolosis due to *F. hepatica* and *F. gigantica* in Ethiopia has long been known and its prevalence and economic significance has been reported (Tadele and Worku, 2007).

Bovine fasciolosis is an economically important parasitic disease of cattle in tropical and subtropical countries responsible for considerable economic losses in the cattle industry, mainly through mortality, morbidity, reduced growth rate, condemnation of fluke infected liver, increased susceptibility to secondary infections and expense due to control measures. Production loss in livestock industry is estimated at more than 90 million USD annually (Rahmeto et al., 2010).

Although a number of studies have been undertaken with regard to abattoir-based prevalence and evaluation of the economic loss due to fasciolosis in different parts of Ethiopia, very little has been done in Awi zone of the Amhara regional state of the country, and particularly Dangila district. Therefore, this study was conducted with the aims to determine the prevalence of fasciolosis and *Fasciola* species among cattle slaughtered at Dangila municipal abattoir, compare the diagnostic efficiencies of fecal versus post mortem examinations and assess direct and indirect economic losses caused by fasciolosis in the study area.

MATERIALS AND METHODS

Study area

The study was conducted at Dangila town, located under the administration of Awi zone in Amhara region, Ethiopia. Geographically, it is located on elevation of 2200 m.a.s.l with latitude and longitude of 11° 16'N and 36° 50'E, respectively, and it is 485 km southwest from the capital city, Addis Ababa. With Woina Dega temperate climate, the annual average rainfall and temperature amount is 1576 mm and 17°C, respectively. The area experiences two seasons, the rainy season (Keremt) lasts from May to October and dry season (Bega) from November to April. The town has a total land area of 89,007 hectares with total population of 158,688. The livestock population of the area comprises of 926,870 cattle, 11,399 horses, 46,628 sheep, 859,213 poultry, and 11,323 beehives (CSA, 2012).

Study population

The study was conducted on indigenous zebu male cattle slaughtered at Dangila municipal abattoir brought from different localities and livestock markets in their vicinity.

Study design and sampling method

A cross sectional study was conducted from October, 2013 to March, 2014 to determine the prevalence and monetary loss of bovine fasciolosis by using post postmortem examination of liver of each slaughtered animal and coprological examination during ante mortem before the animals were allowed for slaughter. Simple random sampling method was employed among cattle brought into the abattoir for determining the prevalence of fasciolosis in cattle and the magnitude of direct monetary loss due to liver condemnation and indirect carcass weight loss at Dangila municipal abattoir. Information regarding age, body condition and months of the study animals were recorded during ante mortem and post mortem examinations.

Sample size determination

To calculate the total sample size, the following parameters were used: 95% level of confidence interval (CI), 5% desired level of precision and with the assumption of 50% expected prevalence of bovine fasciolosis in the study area the sample sizes were determined using the formula given in Thrusfield (1995).

$$n = \frac{1.96^2 \times Pexp\ (1\text{-}Pexp)}{d^2}$$

Where n = required sample size, P_{exp} = expected prevalence, d^2 = desired absolute precision. Hence, 384 samples were used for the study.

Study methodology

Coprological examination

Ante mortem inspections were conducted on individual animals in motion and at rest, while they were in the lairage. During ante mortem examination, detail records with regard to their ages and body conditions of the animals were performed. The age of the animals was scored according to De-Lahunta and Habel (1986) and the body condition of the animal was scored according to Mari (1989). Following the judgments passed by Food and Agricultural Organization (FAO) (1994), animals fit for human consumption were allowed for slaughter. Prior to slaughter, fecal samples were collected directly from the rectum of each study animal, using disposable plastic gloves and placed in clean universal bottle and each sample was clearly labeled with animal identification, age and body condition score. Fecal samples were preserved with 10% formalin solution to avoid the eggs developing and hatching. In the laboratory, coprology was used to detect the presence of *Fasciola* eggs using the standard sedimentation techniques recommended by Hansen and Perry (1994). Abattoir-based prevalence was used to determine positivity of the animals for the disease, but coprology was used to compare the diagnostic efficiency of the disease with post mortem examination.

Post mortem examination

The prevalence of fasciolosis was conducted by post mortem examination technique from liver parenchyma and major bile ducts to recover the young flukes and adult parasites, respectively. The previously (in ante mortem) identified animals and their livers were carefully supervised and examined, so as to avoid mixing up of the organs to be inspected with the fecal samples. Post mortem examination of liver and associated bile duct was carefully performed by visualization and palpation of the entire organ followed by transverse incision of the organ across the thin left lobe based on routine meat inspection by FAO (2003) in order to confirm the presence of the parasites. Livers were inspected by making multiple deep incisions of the lobes and making a deep cut with a number of small sub cuts. Gall bladders were opened using a knife and thoroughly investigated for the presence of *Fasciola.* For *Fasciola* species identification, one or more samples of the worms were collected from condemned livers which had active infection. Species identification was conducted on recovered *Fasciola* based on morphological features of the agents and classified into *F. hepatica* and *F. gigantica*, mixed infection by both flukes and unidentified or immature forms of liver flukes (Urquhart et al., 1996).

Monetary loss assessment

The total monetary loss due to fasciolosis in cattle slaughtered from the summation of annual liver condemnation cost (direct loss) and cost due to carcass weight reduction (indirect loss) was assessed.

Direct monetary loss

Direct monetary loss was resulted from condemnation of liver affected by fasciolosis. All livers affected with fasciolosis were totally condemned. The annual loss from liver condemnation was assessed by considering the overall annually slaughtered animals in the abattoir and retail market price of an average zebu liver. Annual slaughtered rate was estimated from the retrospective abattoir records of the last three years, while retail market price of an average size zebu liver was determined from the information collected from butcheries in Dangila town. The average numbers of cattle slaughtered in Dangila municipal abattoir were 3500 cattle per year based on three years recorded data while the mean retail price of one liver is 35 Ethiopian birr. The information obtained was subjected to mathematical computation using the formula set by Ogunrinade and Adegoke (1982).

$$ALC = MCS \times M\ LC \times P$$

Where; ALC = annual loss from liver condemnation; MCS = mean annual cattle slaughtered at Dangila municipal abattoir; M LC = mean cost of one liver in Dangila town; P = prevalence of the disease at the study abattoir

Indirect monetary loss

Indirect monetary loss was associated with carcass weight reduction due to fasciolosis. Carcass weight loss in individual cattle due to fasciolosis is 10%, while an average carcass weight of Ethiopian zebu was taken as 126 kg (International Lactation Consultant Association (ILCA), 1992). The mean retail price of one kilogram of beef in Dangila town was taken as 80 Ethiopian birr. The annual carcass weight loss due to bovine fasciolosis was assessed using the following formula set by Ogunrinade and Adegoke (1982).

$$ACW = MCS \times CL \times BC \times P \times 126\ kg$$

Where; ACW = annual loss from carcass weight reduction; MCS = mean annual cattle slaughtered at Dangila municipal abattoir; CL = carcass weight loss in individual cattle due to fasciolosis (10%), BC = average price of 1 kg beef at Dangila town; P = prevalence of the disease at the study abattoir, average carcass weight of Ethiopian zebu = 126 kg.

Data analysis

All raw data generated from this study were coded and entered in MS Excel database system. Using statistical package for social science (SPSS) version 16.0 computer program, data were analyzed. The prevalence of fasciolosis was calculated as the number of infected individuals divided by the number of cattle examined × 100. Categorical data were analyzed with the Pearson's Chi-square (χ^2) test to measure the association between prevalence of the parasite with the potential risk factors as a statistical tool. Identification of the dominant *Fasciola* species was calculated using percentage. For all analysis, $P < 0.05$ was considered as significant differences between the parameters measured. The monetary loss of the disease was analyzed and calculated using the formula set by Ogunrinade and Adegoke (1982).

RESULT

Coprological examination

From a total of 384 fecal samples examined for fasciolosis, 22.14% (85/384) were found to be positive by coprological examination (Table 1). The highest prevalence of bovine fasciolosis was observed during October 30.7% (31/101) and the lowest was seen during March 6.7% (2/30). Statistically analysis showed that there was significant difference ($P < 0.05$) in prevalence of fasciolosis among months during coprological examination (Table 1). The prevalence of fasciolosis among body conditions of the animals was found to be 16.1% (35/218), 27.6% (34/123) and 37.2% (16/43) in good, medium and poor body conditions, respectively. The highest prevalence was observed in poor body condition

Table 1. Prevalence of bovine fasciolosis among months during coprology.

Months	Examined	Positive	Prevalence (%)	χ^2	P-value
October	101	31	30.7	12.217	0.032
November	75	20	26.7		
December	93	19	20.4		
January	58	10	17.2		
February	27	3	11.1		
March	30	2	6.7		
Total	384	85			

Table 2. Prevalence of bovine fasciolosis among body condition during coprology.

Body condition	Examined	Positive	Prevalence (%)	χ^2	P-value
Good	218	35	16.1	12.509	0.002
Medium	123	34	27.6		
Poor	43	16	37.2		
Total	384	85			

Table 3. Prevalence of bovine fasciolosis between ages during coprology.

Age	Examined	Positive	Prevalence (%)	χ^2	P-value
Young	104	24	23.1	0.073	0.787
Adult	280	61	21.8		
Total	384	85			

Table 4. Prevalence of bovine fasciolosis among months during post mortem.

Months	Examined	Positive	Prevalence (%)	χ^2	P-value
October	101	41	40.6	17.990	0.003
November	75	29	38.7		
December	93	26	28.0		
January	58	12	20.7		
February	27	5	18.5		
March	30	3	10.0		

as compared to good and medium body conditions. There was statistically significant association (P < 0.05) in prevalence of fasciolosis among the three body conditions of the animals (Table 2). Based on age of the animal the prevalence of fasciolosis was found to be 23.1% (24/104) and 21.8% (61/280) in young (< 5 years) and adult (> 5 years), respectively. There was no statistical significant association (P > 0.05) in prevalence of fasciolosis between the two age groups (Table 3).

Post mortem examination

Out of 384 cattle slaughtered, 30.21% (116/384) were found to be positive for fasciolosis by post mortem examination (Table 1). The highest and the lowest prevalence of fasciolosis was recorded 40.6% (41/101) and 10.0% (3/30) in October and March, respectively. Statistical analysis showed that there was significant difference (P < 0.05) on prevalence of fasciolosis among different months (Table 4). During post mortem examination, the prevalence of fasciolosis was found to be highest in poor body condition 46.5% (20/43) when compared to cattle with good 22.5% (49/218) and medium 38.2% (47/123) body conditions. Statistical analysis showed that there was significant difference (P < 0.05) in prevalence of fasciolosis among the three body conditions (Table 5). The prevalence of fasciolosis in association with different age groups, the highest prevalence was recorded in young (< 5 years) 32.7%

Table 5. Prevalence of bovine fasciolosis among body condition during post mortem.

Body condition	Examined	Positive	Prevalence (%)	χ^2	P-value
Good	218	49	22.5		
Medium	123	47	38.2	15.338	0.000
Poor	43	20	46.5		
Total	384	85			

Table 6. Prevalence of bovine fasciolosis between ages during post mortem.

Age	Examined	Positive	Prevalence (%)	χ^2	P-value
Young	104	34	32.7	0.091	0.426
Adult	280	82	26.3		
Total	384	116			

(34/104) as compared to adult (> 5 years) 29.3% (82/280) during post mortem examination. Statistical analysis showed that there was no significant difference (P > 0.05) in prevalence of fasciolosis between the two age groups of the animals (Table 6).

Fasciola species identification

Among 116 livers infected by *Fasciola*, *F. hepatica* was found to be the highest prevalence of 42.2% (49/116), while *F. gigantica,* mixed infestation with the two species and immature flukes were found to be 27.6% (32/116), 19.0% (22/116) and 11.2% (13/116), respectively (Table 7).

Table 7. Prevalence of *fasciola* species found among infected livers.

Species	Livers infected	Prevalence (%)
F. hepatica	49	42.2
F. gigantica	32	27.6
Mixed infection	22	19.0
Immature	13	11.2
Total	116	100

Comparison of coprological and post mortem results of fasciolosis

From a total 384 cattle examined for the presence of *Fasciola*, post mortem finding revealed better results of 116 (30.21%) than coprological findings 85 (22.14%) (Table 8)

Monetary loss analysis due to bovine fasciolosis

The average current cost of one liver and one kilogram beef in Dangila town was 35 and 80 ETB, respectively. Based on this information the total annual monetary loss due to fasciolosis was calculated by using the following formula:

Annual loss from liver condemnation (ALC) = MCS × MLC × P

Where; ALC = Annual loss from liver condemnation; MCS = Mean annual cattle slaughtered at Dangila municipal abattoir (3500); M LC = Mean cost of one liver in Dangila town (35 ETB); P = Prevalence of the disease at the study abattoir (30.21%).

ALC = MCS × M LC × P (31,720 ETB)

Annual loss from carcass weight reduction (ACW) = MCS × CL × BC × P × 126 kg

Where; ACW = annual loss from carcass weight reduction; MCS = mean annual cattle slaughtered at Dangila municipal abattoir (3500), CL = carcass weight loss in individual cattle due to fasciolosis (10%), BC = average price of 1kg beef at Dangila town (80 ETB); P = prevalence of fasciolosis at Dangila abattoir (30.21%), average carcass weight of Ethiopian Zebu = 126 kg

ACW = MCS × CL × BC × P × 126 kg (913,550 ETB)

Therefore, the total annual monetary loss due to fasciolosis at Dangila municipal abattoir was found to be the summation of both annual loss from liver condemnation and annual loss from carcass weight reduction 945,270 ETB (48,432 USD).

DISCUSSION

The overall prevalence of fasciolosis found in the present

Table 8. Overall prevalence of bovine fasciolosis between coprology and post mortem.

Examination techniques	Examined	Positive	Prevalence (%)
Coprology	384	85	22.14
Post mortem	384	116	30.21

study was higher during post mortem examination (30.21%) than coprological findings (22.14%). This finding was in line with the prevalence reported by Edilawit et al. (2012) as 25.33 and 15.67% at Wolaita Sodo abattoir and Mulat et al. (2012) as 29.75 and 19.5% at Gondar ELFORA abattoir both in post mortem and coprological examinations, respectively. This may be due to the need of longer period from 8 to 15 weeks after infection for the appearance of *Fasciola* eggs in the feces. Furthermore, the detection of *Fasciola* eggs and the appearance of the disease in some areas were difficult to detect during the prepatent period because eggs are expelled intermittently depending on the evacuation of the gall bladder and life cycle of *Fasicola* (Radostits et al., 2007). Coprological examination also includes numerous steps that increase the chance of losing eggs, as demonstrated by the lower number of positive result recorded in this work, as a result of eggs may remain in the debris while filtering the feces through gauze or may get fixed on the bottom and wall of the container and within the pipette when taking the sediment for microscopic observation.

The prevalence of bovine fasciolosis during coprological examination (22.14%) in this study was lower than the findings of 41.41% in and around Woreta, North Western Ethiopia by Tsegaye et al. (2012); 34.9% by Bekele et al. (2014) in Lemo district, Southern Ethiopia and 31.5% by Ayalew and Endalkachew (2013) at Bahir-Dar municipal abattoir, Northern Ethiopia. The reason behind the variation with the above reports may be due to the expansion of animals' health post at peasant association level and the intervention of nearby private veterinary drug shop and pharmacies as well as good management practices of the individual owner of the animal. This enables the farmers to have more access for disease control and intervention. Moreover, the variation of season when the research conducted may be the probable reason for the difference prevalence with the above findings.

In the present study, the prevalence of bovine fasciolosis during post mortem examination was found to be 30.21%. This result was in agreement with the findings of Bekele et al. (2014) with prevalence of 30.5% in Hossana municipal abattoir. While this finding was lower as compared to the result by Abie et al. (2012) with prevalence of 54.5% in Jimma municipal abattoir. On the contrary, the result was higher than the findings of 25.33% by Edilawit et al. (2012) in Wolaita Sodo, Ethiopia. The reason behind the variation of the present results with the above findings might be attributed to the differences in ecology of the study areas, management systems of animals, sample size as well as the present study were conducted during the dry period of the year when the infection rates of fasciolsis is expected to be low, since one of the most important factors that influence the occurrence of fasciolosis in an area is the availability of a suitable habitat for the snail intermediate hosts and essential for the development of fluke eggs, miracidia searching for snails and dispersal of cercariae (Urquhart et al., 1996).

The present study according to age groups had no significant difference ($P > 0.05$). This showed that age groups have no effect for the presence or prevalence of fasciolosis; hence, both animals were equally exposed to infection, but the prevalence of fasciolosis in young was higher than adults both in coprology and post mortem examinations in the study area, which is in line with the finding by Bekele et al. (2014) resulting highest prevalence in young than adult animals. This is clearly justified that the decrease in infection rate (prevalence) as age increase is the result of acquired immunity which is manifested by humeral respond and tissue reaction in bovine liver due to previous challenge. The increased resistance (low prevalence) as age increase is most likely related to the high level of tissue reaction seen in bovine liver, server fibrosis which impedes the passage of immature fluke, acquired resistance, thickening, stenosis and calcification of bile ducts, assumed unfavorable site for adult parasites and consequently fasten their explosion. On the contrary, results indicating inverse correlation of prevalence rate and age of cattle were reported by Tsegaye et al. (2012) in young and adult with prevalence of 40.0 and 42.24%, respectively.

The monthly/seasonal variation in the prevalence of fasciolosis has been studied for 6 dry months in the study area. It was difficult to indicate the effect of seasonal variation on the prevalence of bovine fasciolosis since the study period was too short without incorporating wet months of the season. An accurate description of seasonal occurrence requires long term epidemiological investigation over several years. Even though the study period is short and in dry season, the highest prevalence of fasciolosis was encountered in October and November, while lower prevalence in February and March both in coprology and post mortem examinations with a statistical significant difference ($P < 0.05$). This result was in line with the report of Edilawit et al. (2012) and Yohannes and Abebaw (2012) who showed the highest prevalence during October and November. The probable justification behind it could be, since the wet ecological conditions still prevailed in October and November, it favors the bionomic requirements for breeding of the *Lymnaea* snails and development of the interamolluscan stages of flukes often reaches the optimum threshold during the wet months of the year. During the dry periods, breeding of snails and development of the larval flukes slow down or stops completely and snails undergo a state of aestivation (Yilma and Malone, 1998).

Species identification on 116 *Fasciola* infected livers, *F. hepatica* was found to be the most prevalent (42.2%) as compared to *F. gigantica* (27.6%), mixed infection by both species (19.0%), and immature flukes (11.2%). This finding is in agreement with reported infection rate of cattle with *F. hepatica* (63.89%), *F. gigantica* (24.07%) and mixed (16.5%) by Tadele and Worku (2007), *F. hepatica* (65.4%), *F. gigantica (*36.0%), mixed infection (11.5%) and immature *Fasciola* species (10.1%) by Abie et al. (2012). Furthermore, it is in agreement with the highest prevalence of *F. hepatica* findings by Mihreteab et al. (2010), Rahmeto et al. (2010), Edilawit et al. (2012) and Bekele et al. (2014). This highest prevalence of *F. hepatica* species might be associated with the existence of favorable ecological biotopes for the snail, *Galba (Lymnaea) truncatula.*

The association between the prevalence of fasciolosis and body condition of the animals was found to be statistical significant. The present study was revealed highest prevalence in poor body condition 46.5 and 37.2% both in post mortem and coprological examinations, respectively as compared to good and medium body conditions. In support of this finding, a study was conducted in Adwa, Ethiopia by Mihreteab et al. (2010), in Wolaita Sodo, Ethiopia by Edilawit et al. (2012); in Jimma, Ethiopia by Abie et al. (2012) and in Hossana, Southern Ethiopia by Bekele et al. (2014) revealed highest prevalence in poor body conditions of cattle than good and medium body conditions. The probable reason could be due to the fact that animals with poor body condition are usually less resistant and are consequently susceptible to various diseases including fasciolosis and due to reduced performance of the animals created by luck of essential nutrients and poor management by the animal owner.

The direct monetary loss as a result of condemnation of liver of cattle and indirect monetary loss due to carcass weight reduction incurred during this study was estimated to be 31,720 Ethiopian birr (ETB) and 913,550 ETB per annum, respectively. Therefore, the total annual monetary loss due to fasciolsis in the study abattoir is the summation of losses from organ condemnation and carcass weight reduction which is 945,270 ETB (48,432 USD) per annum.

The present finding was higher than the financial losses reported by Mihreteab et al. (2010) 4,672 USD at Adwa municipal abattoir, Rahmeto et al. (2010) 106,400 ETB at Hawassa municipal abattoir. On the contrary, the result was lower than the findings of Edilawit et al. (2012) 1,574,482 Ethiopian birr (87,471 USD) per annum in Wolaita Sodo and Abie et al. (2012) 2,570,396 Ethiopian birr (151,200 USD) per annum in Jimma municipal abattoir. This difference in the estimated monetary losses could be attributed to the increase in the price of liver and meat in the global market in general and in Ethiopia in particular.

Conclusion

Bovine fasciolosis is an economically important parasitic disease of cattle in tropical and subtropical countries responsible for considerable economic losses in the cattle industry, mainly through condemnation of fluke infected liver. Although moderate prevalence of bovine fasciolosis was recorded in the study area, the prevalence was significantly affected by body condition and months of the year and they were identified as important risk factors for the occurrence of fasciolosis in cattle.

Higher prevalence of bovine fasciolosis was recorded in poor than good body conditions of the animal. *F. hepatica* was found to be the predominant fasciola species causing bovine fasciolsis in the study area. There was substantial agreement between fecal examination and liver inspection in the diagnosis of fasciolosis, indicated that coprological examination for parasite eggs has significant limitation in detecting the presence or absence of fasciolosis, while examination of the liver of animals during post mortem is the most reliable method to detect fluke infection. The total annual monetary losses due to bovine fasciolosis in the study abattoir from liver condemnation (direct loss) and carcass weight reduction (indirect loss) was estimated to be 945,270 Ethiopian birr, indicating that fasciolosis causes significant losses to economy of the study area in particular and the country in general.

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