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Review

A review on trypanocidal drug resistance in Ethiopia

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Trypanosomosis is a major constraint to livestock production in sub Saharan Africa. The distribution of the disease is influenced by the existence of tsetse and biting flies. Tsetse transmitted trypanosomosis is encountered in many part of Ethiopia. Trypanocidal drugs remain the principal method of animal trypanosomosis control in the country. However, there is growing concern that their future effectiveness may be severely reduced by widespread drug resistance. Because it is very unlikely that new trypanosomal drugs will be released on to the market in the future, it is essential to maintain the efficacy of the currently available drugs. So proper detection methods of drug resistance by test in ruminants, in mice, *in vitro* and molecular tests, and followed by the right techniques on the delay of the development of drug resistance like reduction in the number of treatments, avoidance of under dosage, use of national drug police and if once resistance present allowing integrated control measures such as reducing vector numbers to reduce the number of drug treatments will be of great importance.

Kew words: Africa Ethiopia, Trypanocidal drugs, Trypanosomosis.

INTRODUCTION

Trypanosomosis in domestic livestock causes a significant negative impact in food production and economic growth in many parts of the Africa, particularly in Sub-Saharan Africa (Taylor, 1998). It has greatly hampered people and animals settlement in a considerable part of the Africa. Trypanosomosis that occurs in Africa cover one third of the continent is arguably the most significant disease (ILRAD, 1994). Therefore, it remains the major important constraint to livestock production on the continent. The wide occurrence of this disease in people and their livestock

retards agricultural and economic development in Africa, and 160 million estimated cattle population are at risk from trypanosomosis (ILRAD, 1994). Trypanosomiasis is controlled either by controlling the vector (Glossina) or by controlling the parasite, or a combination of both. Over the years, a large arsenal of vector-control tools has been developed. Nevertheless, the control of animal trypanosomiasis in poor rural communities has continued to rely heavily on the use of trypanocidal drugs. This is not surprising considering the private nature of such treatments and the difficulties in maintaining cleared

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flies. Only a small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use and new compounds are unlikely to become available in the absence of barriers to re-invasion of tsetse available in the near future (Barrett, 2004).

Geerts and Holmes (2004) estimated that in Africa, 35 million doses of veterinary trypanocidal drugs are administered each year with isometamidium chloride (ISMM), ethidium bromide (EtBr) and diminazene aceturate (DA) estimated to represent 40, 26 and 33%, respectively, of the total trypanocidal drug market by value (Sones, 2001). ISMM is mainly used as a prophylactic drug and provides on average 3 months' protection (2 to 22 weeks) against trypanosome infection. DA has only therapeutic properties and EtBr has limited prophylactic properties, and is mainly used as a therapeutic agent (Holmes et al., 2004).

Considering the well known mutagenic properties of EtBr, this drug should ideally be removed from the drug market, but in practice it is still widely used in many countries. Removing this drug from the market would not jeopardize the treatment of animal trypanosomiasis because it can be replaced either by DA for curative purposes or by ISMM for prophylactic purposes. When trypanosomes are resistant to ISMM, EtBr will be ineffective as cross-resistance is observed between the two drugs (Peregrine et al., 1997; Van den Bossche et al., 2000; Delespau et al., 2002).

The total value of the market for trypanocides for African farmers is estimated at US\$30 million (Ismail et al., 2004) but this is considered insufficient by pharmaceutical companies to justify investment in the development of new drugs (Gall et al., 2004), therefore the challenge remains to make optimal use of existing drugs. The repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance in the target organisms. This also occurred with the trypanocidal drugs, such as ISMM, the homidium salts and diminazene aceturate, which were introduced during the 1950s; the first reports of acquired resistance were published during the 1960s (Finelle and Yvone, 1962; Jones-Davies, 1967; Jones-Davies and Folkers, 1966). Treatment and prevention of bovine trypanosomiasis rely essentially on three drugs, namely: homidium, diminazene and isometamidium. However, in many parts of Africa all of these trypanocides are gradually losing their efficacy due to drug resistance. Experimental studies have demonstrated the occurrence of resistance in trypanosomes to both diminazene and isometamidium fortunately, trypanosomes are usually not resistant to both diminazene and isometamidium at the same time (Machila et al., 2001). Thus, these two compounds have been termed a sanative pair, since in instances of resistance to one drug application of the other one will usually control the disease. Nevertheless, recent reports on multiple drug-resistances in Burkina Faso and Ethiopia suggest that the concept of sanative

pairs might no longer always be valid (Afewerk et al., 2000).

Ethiopia, as part of the African continent shares a substantial loss from the disease. Apart from the cyclical transmission of trypanosomiasis by the *Glossina* species, it is highly considered that mechanical transmission significantly affects livestock productivity in many parts of the world including Ethiopia (Abebe and Jobre, 1996). However, information on prevalence of non-cyclically transmitted trypanosomiasis in domestic animals, the vectors involved and the drug sensitivity of the trypanosome species in Ethiopia is scanty and sufficient data in a compiled form is not available (Alekaw, 2004). The situation of African Animal Trypanosomiasis (AAT) has become even worse due to the fact that some drugs have been abandoned due to resistance.

Practically, only ethidium bromide (Homidium) and DA (Berenil) are still available for therapy and ISMM (Samorin, Trypanidium) for prophylaxis (Holmes, 2004; Geerts et al., 1997). The inevitable outcome of continued use of the same compounds for decades has resulted in drug resistance that has been largely responsible for the frequently observed chemotherapeutic failures (Moti et al., 2010). In Ethiopia, the prevalence of mechanically transmitted *Trypanosoma vivax* and *Trypanosoma evansi* reported by the various workers has indicated their wider distribution in the country and the disease impact due to both parasites is substantial. The presence of mechanical vectors, existence of reservoir hosts, and the involvement of wider host range in parasites, the various agro-climatic zones and the poor veterinary infrastructure would undoubtedly ensure the existence of both *T. vivax* and *T. evansi* in Ethiopia.

In Ethiopia, trypanosomiasis is one of the major constraints to cattle production with direct and indirect economic losses (Abebe and Jobre, 1996). Effective vector control methods are available (Bauer et al., 1995). However, in Ethiopia, increasing costs and other problems of initiating and maintaining tsetse control campaigns have led the livestock sector to be completely reliant on the use of trypanocidal drugs. Therefore, the objective of this paper is to over review on trypanocidal drug resistance in Ethiopia.

STRATEGIES FOR TRYPANOCIDAL DRUG USAGE

Routine block treatments

These are generally carried out using prophylactic drugs, notably isometamidium chloride, at predetermined intervals based on the perceived duration of prophylaxis (0.5 to 1 mg/kg BW, intramuscularly). All animals in a herd may be treated or treatment may be targeted at a particular group of valuable or at risk animals. When routine block treatment with isometamidium is practiced, it is recommended that once a year, the animals are

separately treated with diminazene in order to delay the development of drug resistance following the concept of sanative pair (Whiteside, 1960).

Strategic block treatments

These are generally carried out using prophylactic drugs, though curative drugs may also be used. All animals in a herd, or particularly valuable or at risk stock, are treated when challenge, as measured by the number of animals succumbing to infection, reaches a predetermined threshold (Holmes et al., 2004)

Monitoring and treatment of individual infected animals

Cattle are monitored using standard parasitological methods. Treatment of infected animals is generally conducted using a therapeutic drug, usually diminazene aceturate (3.5 to 7 mg/kg BW, intramuscularly) (Eisler et al., 2001).

MODE OF ACTION OF TRYPANOCIDAL DRUGS

Despite the fact that chemotherapy is the major means of disease control, development of new antitrypanosomal drugs has been more or less static over the last three decades, due to lack of interest by the pharmaceutical industry to invest into research and development of antitrypanosomal drugs (Gutteridge, 1985). Consequently, this has been a major stimulus for intensive research into the few existing drugs; and in the recent past, considerable body of knowledge has emerged on a number of important aspects, such as drug disposition, mechanisms of action, resistance and toxicity. The three antitrypanosomal compounds upon which treatment and prophylaxis of cattle trypanosomosis currently depends are isometamidium chloride, homidium chloride or bromide and diminazene aceturate. Whereas, quinapyramine, suramine and melarsomine are primarily used as therapeutic drugs for infections caused by *T. evansi* in equidae, camels and buffaloes, although quinapyramine is also used for prophylactic purpose (Williamson, 1970).

Diminazene aceturate

Diminazene binds to trypanosomal kinetoplast DNA (Newton, 1972; MacAdam and Williamson, 1972). This binding does not occur by intercalation (Newton, 1972) but via specific interaction with sites rich in adenine-thymine (A-T) base pairs (Newton, 1972; Brack and Delain, 1975). Non-intercalative binding of diminazene to

DNA, with strong affinity for A-T base-pair regions, has similarly been demonstrated *in vitro*, using DNA obtained from various sources (Lane et al., 1991). Such studies have shown that the molecule binds with higher affinity to 5'-AATT-3' than to 5'-TTAA-3' regions of DNA. Through this specific interaction in trypanosomes, diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Newton, 1972; Brack and Delain, 1975). In other work, Shapiro and Englund (1990) have shown that diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes. Thus, inhibition of DNA replication may also occur via this intercalation. The rate of excretion of the different compounds is known to affect their activity. Diminazene, which is rapidly excreted, is used only for its therapeutic effect. Dimidines accumulate in the liver for months, like wise in the kidney and the adrenal glands respectively (Biobaku et al, 2008).

Isometamidium

The primary mode of action currently considered to account for the molecular mechanisms of antitrypanosomal activity of phenanthridinium drugs is blockade of nucleic acid synthesis through intercalation between DNA base pairs (Wagner, 1971), inhibition of RNA polymerase (Richardson, 1973), DNA polymerase and incorporation of nucleic acid precursors into DNA and RNA (Lantz and Van Dyke, 1972). Other biochemical reactions that may account partly to their effects include modulation of glycoprotein biosynthesis, lipid metabolism (Dixon et al., 1971), membrane transport (Girgis-Takla and James, 1974) and selective cleavage of kinetoplast DNA minicircles. The mechanism that is considered primary is blockade of nucleic acid synthesis, which does not explain the basis of their selective toxicity. However, there are a number of biochemical peculiarities that have been demonstrated in trypanosomes that appear to be candidate targets for drug modulation, and that might explain the basis of selective toxicity (Opperdoes, 1985). Generally it inhibits DNA synthesis in a similar manner as diminazene aceturate, it modifies the mitochondrial membrane, it modifies the glycoprotein structure in surface of the endoplasmic reticulum and Isometamidium is slowly excreted, and is the most effective prophylactic compound currently available (Kinabo and Bogan, 1988).

Homidium salt

Pentamidine, diminazene aceturate (Berenil), isometamidium chloride (Samorin), and ethidium bromide, which are important antitrypanosomal drugs, promote linearization of trypanosoma minicircle DNA (the principal component of kinetoplast DNA, the

mitochondrial DNA in these parasites) (Delespaux et al., 2002). This effect occurs at therapeutically relevant concentrations. The linearized minicircles are protease sensitive and are not digested by lambda exonuclease (a 5' to 3' exonuclease), indicating that the break is double stranded and that protein is bound to both 5' ends of the molecule. The cleavage sites map to discrete positions in the minicircle sequence, and the cleavage pattern varies with different drugs. These findings are characteristic for type II topoisomerase inhibitors, and they mimic the effects of the antitumor drug etoposide (VP16-213, a semisynthetic podophyllotoxin analog) on *Trypanosoma equiperdum* minicircles. However, the antitrypanosomal drugs differ dramatically from etoposide in that they do not promote detectable formation of nuclear DNA-protein complexes or of strand breaks in nuclear DNA. Selective inhibition of a mitochondrial type II topoisomerase may explain why these antitrypanosomal drugs preferentially disrupt mitochondrial DNA structure and generate dyskinetoplastic trypanosomes (Shapiro and Englund, 2003).

MECHANISM OF ANTITRYPANISOMAL DRUG RESISTANCE

The discovery of trypanocidal drugs with preventive action raised high hopes that their use would make it possible to turn subtropical Africa into a flourishing livestock production area. It must be admitted that most of these hopes have not been realized. Although, these drugs do provide protection, which in some conditions may last up to six months, all of them frequently give rise to the formation of drug-resistant trypanosome strains. This drug resistance occurs when the trypanosomes are in contact with a trypanocide administered in a subcurative dose insufficient to ensure the destruction of the parasites (Das et al., 2004). This situation may be due to one or more of the following factors: the application of insufficient doses, due in particular to underestimating the weight of animals; the formation of abscesses followed by partial rejection of the drug; a cyst-forming reaction which prevents the diffusion of the product; preventive treatments at too long or irregular intervals; halting the application of trypanoprophylactics while the animals are still exposed to the risk of infection, and the occasional use of preventive drugs in curative treatments (Rowlands et al., 1994).

An understanding of the mechanisms of drug resistance by trypanosomes, among others, is important as it can lead to the identification of potential and novel drug targets, and provide direction to how new chemotherapeutic strategies can be used to reduce development of resistance. In the latter instance rationale for combinations of existing drugs to increase therapeutic activity, decrease clinical toxicity and potentially reducing the frequency of the emergence of drug resistance

(Barrett and Fairlamb, 1999) can be identified. Trypanocidal drug resistance could be innate, such as in resistant individuals without previous exposure to the particular drug, or acquired (induced) as a result of drug exposure/pressure, cross-resistance or sometimes by mutagenesis (ILRAD, 1990). Reduction in drugs accumulation by the target cell or organism and diminished drug activity in immunosuppressed animals can contribute to the emergence of drug resistance (Frommel and Balber, 1987).

Furthermore, some trypanocidal drugs are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure (Hayes and Wolf, 1990). Taking into account the high basic mutation rate in trypanosomes, which is estimated at 10^{-9} per base pair per cell generation in *Trypanosoma brucei* the effects of this phenomenon should not be underestimated.

Diminazine acetate

Although, diminazene probably exerts its action at the level of the kinetoplast DNA, this has not been proven *in vivo* and other mechanisms of action cannot be excluded. Similarly, the molecular basis of resistance to diminazene in trypanosomes is not clear (Berger et al., 1995) which showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei brucei* owing to alterations in the nucleoside transporter system (P2).

However, there might be other resistance mechanisms. Similarly to ISMM, contradictory reports have also been published on the stability of resistance to diminazene. Mulugeta et al. (1997), however, showed that the phenotype of multiple drug-resistant *Trypanosoma congolense* remained stable over a period of four years. In conclusion, it is clear that much more work is required in order to elucidate the mechanism of resistance to the three currently available trypanocidal drugs. Such studies, as well as being of great value in their own right, may also provide novel methods for the detection of drug-resistant trypanosomes in the future.

The same is true for the genetics of drug resistance in trypanosomes, which distinguish three major types of genetic change that are responsible for acquired drug resistance: mutations or amplifications of specific genes directly involved in a protective pathway; mutations in genes that regulate stress-response processes and lead to altered expression of large numbers of proteins; and gene transfer. Gene amplification under conditions of drug pressure is well known in *Leishmania* spp. and has also been demonstrated in trypanosomes, but until now there is no evidence that this occurs in the latter parasites as a mechanism of drug resistance (Ross and Sutherland, 1997).

The current possibilities to insert or delete genes will certainly lead to a better insight into the resistance

mechanisms (Ten Asbroek et al., 1990; Gaud et al., 1997). Other aspects, such as the stability of drug resistance, its mono- or polygenic nature, dominance or recessiveness, also need to be examined, because of their far-reaching impact on the control of resistance.

Isometamidium

The main mode of action of ISMM was the cleavage of kDNA-topoisomerase complexes. This explanation was supported by Wilkes et al., (1997) who showed that the trypanosome kinetoplast is the primary site of ISMM accumulation. The mechanism of resistance to ISMM, however, is less clear. Decreased levels of drug accumulation have been observed in drug-resistant populations of *T. congolense* (Sutherland et al., 1991), and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 2004). Recently, Mulugeta et al. (1997) showed that the maximal uptake rates (V_{max}) of ISMM in resistant *T. congolense* were significantly lower than in sensitive populations. It remains to be shown whether this is caused by a decreased number of protein transporters of ISMM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISMM by *T. congolense* is yet to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* more recently, changes in mitochondrial electrical potential have been demonstrated in ISMM-resistant *T. congolense* by Wilkes et al. (1997). Although, contradictory observations have been reported on the genetic stability of ISMM resistance, recent field observations in Ethiopia, based on cloned populations, showed that the drug-resistant phenotype of *T. congolense* had not altered over a period of four years (Mulugeta et al., 1997).

It remains unclear whether the ISMM-resistance phenotype is the consequence of reduced uptake or increased efflux of the drug. In one study, sensitive strains were heterozygous for the GAA codon insertion, whereas most resistant strains were homozygous for the same trait (Delespaux et al., 2002). The fact that the sensitive isolates already seem to carry a recessive resistance allele is consistent with the selection of an existing influx transporter expressed at a lower level or with decreased affinity for ISMM through loss of heterozygosis. Alternatively, the resistance allele could encode a mitochondrial efflux pump with increased affinity for ISMM. However, such an allele would be expected to be dominant, actively clearing the drug from the kinetoplast. To challenge this model, more isolates should be screened to identify an ISMM-sensitive phenotype for a strain homozygous for the insertion. A combined mechanism of reduced uptake and increased efflux might also be possible. Three major types of

genetic change that are responsible for acquired drug resistance are identified: mutations or amplifications of specific genes directly involved in a protective pathway; mutations in genes that regulate stress-response processes and lead to altered expression of large numbers of proteins; and gene transfer. Gene amplification under conditions of drug pressure is well known in *Leishmania* spp. and has also been demonstrated in trypanosomes, but until now there is no evidence that this occurs in the latter parasites as a mechanism of drug resistance under field conditions (Hayes and Wolf, 1990).

Homidium salts

Although, their mutagenic activity has been known for a long time (MacGregor and Johnson, 1977), homidium chloride and especially homidium bromide or ethidium are still widely used as trypanocidal drugs. The mechanism of their antitrypanosomal action is not well understood. However, it has been shown that the drugs interfere with glycosomal functions, the function of an unusual adenosine monophosphate-(AMP) binding protein, trypanothione metabolism and the replication of kinetoplast minicircles (Wang, 1995). The mechanism of resistance by trypanosomes to these drugs is unknown. There are indications, however, that it is similar to that described for ISMM.

DETECTION OF ANTI TRYPANOSOMAL DRUG RESISTANCE

Several methods have been described to identify drug resistance in trypanosomes (Peregrine, 1994, 1996). At present, four types of technique are commonly used to identify drug resistance: tests in ruminants; tests in mice, *in vitro* assays and now a day molecular detection also possible. None of these is, however, an ideal test and other tests are still in the phase of development or validation. The advantages and disadvantages of each of the different techniques are briefly summarized in the following sections.

Detection in ruminant

The tests commonly consists of infecting a group of cattle or small ruminants with the isolate under investigation and later, when they are parasitemic, treating them with various dosages of trypanocidal drugs (Delespaux, 2000). It is preferable to use at least three animals in each group because it has been shown that results obtained after inoculation and treatment of one animal are not always reliable. The animals are regularly monitored over a period of 100 days to determine the

efficacy of standard drug doses in terms of their ability to precede permanent cure. For these studies, the cattle or small animals are kept in fly proof accommodation or in none tsetse area in order to eliminate the risk of reinfection during the study. A variation on this technique is to inoculate blood from several different infected cattle into single recipient calf (Holmes et al., 2004).

Detection in mice

Tests in mice can be used as a single dose test or as a multi dose test. In the later case, the objective is to obtain more detailed information by determining the CD50 or CD80 values (curative dose that gives cure in 50 or 8% of animals) for a given trypanocidal drug. In the case of a single test, a large number of trypanosome isolates is tested at a single discriminatory dosage of 1 mg/kg for ISMM and 20 mg/kg for DA (Eisler et al., 2001). The advantage of the mouse test is that it is cheaper than the test in ruminants. However, it presents several disadvantages; firstly, most *T. vivax* isolates, and also some *T. congolense* isolates do not grow in mice and for that reason, research on *T. vivax* isolates in particular has been hampered. Secondly, higher dose of drug must be used in mice in order to obtain results comparable to those from cattle because of the vast difference in metabolic size, in spite of the fact that there is reasonable correlation between drug sensitivity data in mice and cattle. Therefore, results in mice cannot be directly extrapolated to calculate the curative dose to be used in animals. Thirdly, a large number of mice per isolate are required in order to obtain a precise assessment of the degree of resistances. This makes it a rather labour intensive test. Finally the test takes as long as 60 days to evaluate the drug sensitivity of an isolate (Greets and Holmes, 2004).

In vitro assays

For the *in vitro* evaluation of drug sensitivity procyclic, metacyclic or blood stream forms of trypanosomes can be used. The advantage of *in vitro* assays is that large number of isolates can be examined however, there are several disadvantages. The use of metacyclic and blood stream forms is considered more reliable than the use of procyclic forms. Test with metacyclic trypanosomes is considered to generate well with field observations, but it may take up to 40 to 50 days of *in vitro* incubation to generate metacyclic trypanosomes (Gray et al., 1993). *In vitro* cultivation of blood stream forms is only possible using pre adapted lines and not using isolates directly from naturally infected animals. *In vitro* assay are expensive to perform and require good laboratory facilities and well trained staff. In contrast to *T. brucei*, it is very difficult to cultivate *T. congolense* (Holmes et al.,

2004) if techniques can be improved to adapt trypanosomes isolates to grow *in vitro* more rapidly, these may become more popular especially in those laboratory where culture facilities are already established. An interesting alternative is the drug incubation glossina infectivity test in which the trypanosomes are exposed to the drug *in vitro* for a short time, and thereafter fed to tsetse flies to check whether or not they develop into metacyclic forms, this technique distinguish resistant from sensitive isolates and does not require experimental animals, but it does require a ready supply of teneral tsetse flies from an artificially reared colony (Holmes et al., 2004).

Molecular detection drug resistance

Molecular methods for the diagnosis of ISM resistance were recently developed (Delespaux et al., 2005). The first method enables discrimination between ISM-sensitive and ISM-resistant strains of *T. congolense* by *MbolI*-PCR-RFLP (Delespaux et al., 2005). This test is based on the polymorphism observed in a 381 bp fragment in sensitive strains or 384 bp fragments in resistant strains of a putative gene presenting some homologies with an ABC transporter. The second method has been developed to distinguish ISM-resistant from ISM-sensitive strains of *T. brucei* (Afeework et al., 2006). This *Sfa*NI-PCR-RFLP test is based on the polymorphism of a 677 bp fragment of the *TbAT1* gene. The same set of six point mutations could confer resistance to the melarsenoxide cysteamine cymelarsan (an arsenical diamidine) and to ISM (diamidine compound) while the detection of one of these six mutations could enable reliable identification of sensitivity or resistance to ISMM.

CURRENT SITUATION OF DRUG RESISTANCE ON TRYPANOSOMOSIS IN ETHIOPIA

So far, resistance to one or more of the common trypanocidal drugs used in cattle has been reported in at least four regional states (local areas) within the country. But the currently available information on drug resistance is derived from limited number of cases reports, and does not give any indication of the true situation of the resistance in a whole country (region) as systematic surveys have not been fully conducted. Table 1 summarizes the list of published reports in which a number of trypanosomes isolate has been examined. This problem of drug resistance in trypanosomes requires being spreading geographically into many regions in which trypanosomes occur. Additionally, the spread of genetic products, some of which are of doubtful quality, may undermine farmer's confidence using trypanocidal drugs (Holmes et al., 2004). Chemotherapy and chemoprophylaxis are the most practical methods available for the control of animal. Trypanosomiasis, but

Tables 1. Multiple and single trypanocidal drug resistance reported in Ethiopia.

Site of study area	Drug tested	Species of parasites	References	
Oromia Regional state	Gibe valley	ISMM & DA	T.C	Moti et al. (2010)
	Upper didesa	ISMM	T.C, T.V & T.B	Tewelde et al. (2004)
	Gibe valley	ISMM & DA	T.C	Chaka and Abebe (2003)
	Bedele	ISMM & DA	T.C	Chaka and Abebe (2003)
	Gibe valley	ISMM, DA & H	T.C	Mulugeta et al (1997)
Benshangul gumz	Metekel	ISMM & DA	T.C	Afewerk et al (2000)
	Metekel	ISMM & DA	T.C	Afewerk (1998)
SNNPRS	SODO	ISMM & DA	T.C	Chaka and Abebe (2003)
	Arbaminch	ISMM & DA	T.C	Chaka and Abebe (2003)
	Omo valley	ISMM & DA	T.C	Ademe (1998)
Tigray	Tselemt	DA & ISMM	T.V	Desalgn et al. (2010)

ISMM (Isometamidium), DA (diminazine Aceturate, H (homidium salt), TC (trypanosoma congolense, T.V (trypanosome vivax, T.B (trypanosome brucei) and SNNPRS (South Nation and Nationality People Regional State).

their effectiveness is being eroded by the emergence resistant trypanosomes. Unfortunately, farmers can purchase a variety of trypanocidal drugs in most markets, although all trypanocidal drugs are supposed to be imported and supplied through the Ministry of Agriculture. The widespread use, the irregular use of prophylactics drugs, their discontinuation while livestock remain at risk, the high incidence of trypanosomiasis and misuse of drugs has contributed to the development of drug resistance in the population of *T. congolense* parasites (Afewerk et al., 2000; Ermiyas and Getachew, 2001). The magnitude of drug resistant trypanosomes across Ethiopia is not well documented. However, some study on a few isolates of *T. congolense* indicated the potential risk for the future in the greater part of tsetse infested areas, where the proportional infection rate of cattle by *T. congolense* is increasing (Abebe and Jobre, 1996) and where dependence on regular drug treatment for trypanosomiasis control, which is a common practice now in Ethiopia, may lead to the risk of major drug resistance development.

CONCLUSION AND RECOMMDETION

The great potential of livestock to rural farmers, in Ethiopia, can only be exploited if trypanosomiasis and the arising appearance of drug resistance are controlled. Chemotherapy and chemoprophylaxis are the most realistic method accessible for the control of animal trypanosomiasis. However, the increasing trend of drug resistant strains of trypanosomes is a serious threat to cattle production in Ethiopia. Unfortunately, farmers can purchase a variety of trypanocidal drugs in most markets, although all trypanocidal drugs are supposed to be

imported and supplied through the Ministry of Agriculture. Exposure of parasites to sub therapeutic drug concentrations, resulting from under dosing and uncontrolled use of trypanocidal drugs, and the lack of proper diagnosis, are considered the major causes of increasing drug resistance in Ethiopia. Therefore, there is an urgent need for detailed experimental work in the field to monitor the development of drug resistance in tsetse-infested areas of Ethiopia.

Furthermore, strict supervision on the usage of trypanocidal drugs should be implemented in order to minimize the misuse. More attention should be given to the adoption of an integrated trypanosomiasis control strategy, involving the vector as well as the parasite. Moreover, effective use of available drugs is crucial. Drug resistance in trypanosomiasis is a serious problem, this situation should be put under continuous study and when detected immediate reversal mechanism have been employed. Therefore, more attention should be given to adopting an integrated disease management strategy involving the vector as well as the parasite such strategies should be economically feasible, socially acceptable and sustainable and environmentally friendly. Legislative reinforcement by way of elaborating a national drug use policy is required to address the indiscriminate drug usage in Ethiopia.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

A study on correlation of malaria infection with A, B, O, RH blood group system

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The aim of this study was to find correlation of malaria infection with A, B, O and Rh blood grouping. A total of 200 blood samples were collected from suspected malaria cases out of which 40 samples were positive and 160 samples were negative for malaria. ABO and Rh blood grouping was done using Eryscreen®. Maximum numbers of malaria positive were seen in blood group 'O' positive followed by 'B' positive, 'A' positive and 'AB' positive. This study suggests that the person having blood group 'O' positive are more prone to malarial infection in endemic areas.

Key words: ABO and Rh blood group, malarial parasites, malaria, microscopy.

INTRODUCTION

Malaria is caused by an obligate, intracellular protozoan parasite of the genus *Plasmodium*. Of the four species that infect humans (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*), *P. falciparum* is responsible for high mortality (Pathirana et al., 2005). The virulence of *P. falciparum* has been associated with the capacity of the infected red blood cells (RBCs) to adhere to uninfected RBCs, leading to rosetting of cells (Carlson et al., 1990; Ringwald et al., 1993). Previous studies have implicated the ABO blood group type in rosetting (Thakur and Verma, 1992). Blood group antigens A and B are trisaccharides attached to a variety of glycoproteins and glycolipids on the surface of erythrocytes, and these trisaccharides are thought to act as receptors for rosetting on uninfected erythrocytes and bind to parasite rosetting ligands such as PfEMP-1 and sequestrin (Martin et al., 1979; Ockenhouse et al., 1992). However, blood group antigens A and B are not

expressed in blood group O individuals. As a result, rosettes formed by blood group O are suggested to be smaller and easily disrupted than rosettes formed by blood group A, B or AB erythrocytes (Daniel, 2005; Barragan et al., 2000).

The association of genetic markers with malaria has been the subject of numerous investigations, since the protection afforded by sickle-cell hemoglobin against infection by *falciparum* malaria parasite. A broad range of available evidence suggests that the origin, distribution and relative proportion of ABO blood groups in humans may have been directly influenced by selective genetic pressure from *Plasmodium falciparum* infection (Christine et al., 2007). Clinical reports of ABO blood groups and *P. falciparum* infection, reveals a correlation between disease severity and ABO groups. However, several studies undertaken have been unable to link ABO blood groups to the incidence of malaria or to the repeat attacks

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of malaria (Facer and Brown, 1979; Singh et al., 1995). Recent studies of the pathogenesis of malaria have shown that parasite triggered red blood cell rosette formation is associated with the severity of clinical disease and malaria (Treutiger et al., 1992; Pathirana et al., 2005). Rosetting was established as a *P. falciparum* virulence factor, the expression of which is modified by a variety of host factors. Anti-rosetting activity, presumably mediated by antibodies, was found in sera from patients in malaria endemic areas, and it was demonstrated that such activity was more abundant in individuals with uncomplicated malaria than in those with cerebral disease, suggesting that humoral immunity protects against rosette formation in vivo. Erythrocytes from individuals with sickle-cell trait, α - and β -thalassemia trait or with HbE formed smaller and weaker rosettes than normal HbAA red blood cells. Recently, even *P. vivax* infection has been reported with clinical severity. There is a paucity of hospital-based, comparative studies to investigate the relationship between blood group types and severity of malarial infections (Deepa et al., 2011).

The ABO blood groups consist of A, B and H carbohydrate antigens which can regulate protein activities during infection and antibodies against these antigens. A number of studies were conducted to investigate the association between ABO blood group system and some disease conditions. Some of these studies reported significant associations, suggesting that ABO blood groups have an impact on infection status of the individuals possessing a particular ABO blood group (Tewodros et al., 2011).

Aims and objectives

1. To assess the distribution of ABO blood group and their relationship with *P. falciparum* and *P. vivax* malaria among patients attending a tertiary care hospital, Navi Mumbai.
2. To correlate the blood groups and the clinical presentations including outcome in malaria patients.

MATERIALS AND METHODS

Ethical clearance

The study protocol was reviewed and approved by the Ethical Review Committee of Mahatma Gandhi Mission Institute of Health Sciences (Deemed University), Navi Mumbai. Written informed consent was obtained from all study participants and mothers/caretakers of children under 18 who participated in the study after explaining the purpose and objective of the study.

Place of study

The study was conducted in Department of Microbiology, Mahatma Gandhi Mission Medical College and Hospital, Navi Mumbai, Maharashtra, India.

Study area

The study area is highly endemic for malarial infection. During rainy season from July to October the incidence of the disease is much more and people visit the hospital in maximum numbers. Mahatma Gandhi Mission's (MGM) Medical College, Navi Mumbai is having 900 bedded attach tertiary hospital serving both as the tertiary care hospital as well as the MGM Medical College teaching hospital. It is located in the MGM Campus, Plot No. 1 and 2, NH-4 Junction, Mumbai-Pune Express Way, Sector-1, Kamothe, Navi Mumbai, Maharashtra, India. Navi Mumbai located in the eastern trans harbour in Mumbai, Maharashtra, India, which is endemic to malaria. This region has rainy seasons from June to October. However, urban and suburban areas of Navi Mumbai city have several swampy and unpopular slum areas with poor drainage and waste disposal facilities. These in turn provide fertile breeding grounds for the female *Anopheles* mosquito, the vector for *Plasmodium* species, leading to the burden of endemic malaria.

Study participants

A total of 200 blood samples of malaria suspected patients of outpatient department and inpatient department of medicine, tertiary care hospital at Navi Mumbai, India were included in this study.

Parasite density determination

Thick and thin blood film slides were prepared using Jaswant-Singh-Bhattacharji stain and Leishman's stain. The stained slides were examined under a light microscope using 100 \times oil immersions lens. Parasitaemia was calculated per 200 white blood cells (WBC) assuming 8000 WBC/ μ l of blood (Cheesebrough, 1998).

Determination of blood grouping

Blood group determination ABO blood groups were typed by agglutination using Eryscreen[®] (Anti-A, Anti-B and Anti-D) provided by Tulip Diagnostics (P) Ltd., India. Three drops of whole blood were placed in two different places of a grease-free clean glass slide on which a drop of antiserum for blood group A, B and Rh was added. The blood cells and the antigen were mixed with applicator stick. The slide was then rotated to detect for agglutination and the result recorded accordingly (Barragan et al., 2000; Zoysa, 1985).

RESULTS AND DISCUSSION

This prospective and analytical study was conducted to find any correlation of malaria with blood group A, B, O and Rh. A total of 200 samples were included in this study out of which 40 (20%) samples were malaria positive and 160 (80%) samples were malaria negative. Malaria was confirmed by 3 methods light microscopy, Quantitative buffy coat (QBC) test and rapid malarial antigen test. Out of the 38 to 40 samples, 25 (62.50%) samples were *P. vivax*, 10 (25%) (13.16%) samples were *P. falciparum* and 5 (12.50%) samples were mixed species. There was blood grouping for all samples that is, positive as well as negative. Blood group distribution in malaria suspected patients was A positive 56 (28%), B positive 41 (20.5%), AB positive 23 (11.5%), O positive

Table 1. Showing distribution of blood groups in 200 malaria suspected cases.

Blood groups	Distribution n=200 (%)
A positive	56 (28)
B positive	41 (20.5)
AB positive	23 (11.5)
O positive	58 (29)
A negative	8 (4)
B negative	4 (2)
AB negative	2 (1)
O negative	8 (4)

Table 2. Showing malaria and blood groups.

Blood groups	Total no. of samples	Malaria positive	Percentages (%)
A positive	56	9	16.08
B positive	41	9	21.95
AB positive	23	3	13.04
O positive	58	15	25.86
A negative	8	1	12.5
B negative	4	0	0
AB negative	2	0	0
O negative	8	1	12.5

58 (29%), A negative 8 (4%), B negative 4 (2%), AB negative 2 (1%) and O negative 8 (4%). (Table 1).

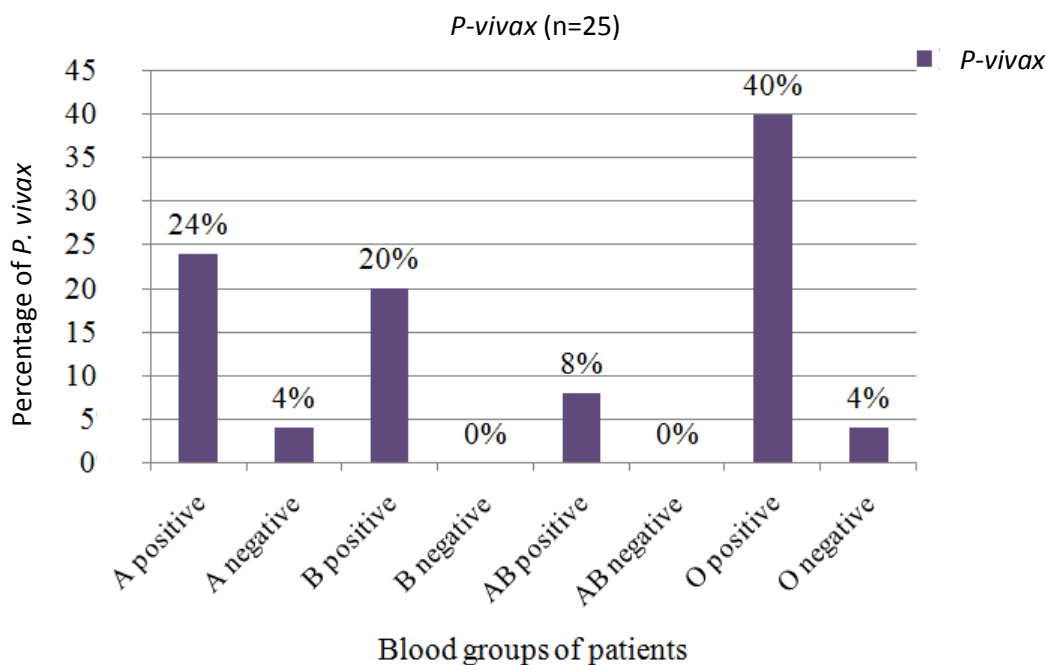
The study suggests that *P. falciparum* malaria patients with blood group O, which is less prone to rosetting have a reduced chance of developing severe falciparum malaria as compared to patients with other blood groups (Zinaye and Beyene, 2010). 'O' group had an advantage over other groups (Deepa et al., 2011). The chance of having a *P. falciparum* infection in patients with blood groups A, B and AB was 2.5, 2.5 and 3.3 times more than individuals showing blood O phenotypes, respectively, blood groups A, B and AB are more susceptible to *P. falciparum* infection as compared with individuals of blood group O (Zerihun et al., 2011). Parasitaemia seemed to be relatively high across all blood groups with groups O and AB subjects apparently recording the highest and least infection rates (Otajewwo, 2013). Patients with O blood group occur most in the clinic, but the prevalence of malaria was highest among those with B blood group (35.3%) and lowest in those with O blood group (17.7%) (Sule et al., 2014). A study reported that individuals of blood group A and B are more susceptible to malaria infection as compared with individuals of blood group O, however, the infection differs due to differential host susceptibility (Gayathri et al., 2013). The respective infective rates were 14.3, 11.1, 13.9 and 0.00% of the blood groups A, B, O and AB. The difference in infection percentage between the various blood groups was,

however, not statistically significant (Muntaka and Opok, 2013) (Table 3). This study showed that maximum numbers of malaria cases were found in blood group 'O' positive that is, 25.86%, followed by B positive that is, 21.95%, A positive that is, 16.08% and AB positive that is, 13.04% (Table 2).

Maximum number of malaria suspected cases was seen in blood group 'O' positive in which 25.86% malaria was positive and 74.14% malaria was negative, followed by blood group 'B' positive in which 21.95% malaria was positive and 78.05% malaria was negative, 'A' positive in which 16.07% malaria was positive and 83.93% malaria was negative, blood group 'AB' positive in which 13.04% malaria was positive and 86.96% malaria was negative, and blood group 'A' negative in which 12.50% malaria was positive and 87.50% malaria was negative and blood group 'O' negative in which 12.50% malaria was positive and 87.50% malaria was negative (Table 2). In *P. vivax*, maximum number of malaria were seen in blood group 'O' positive that is, 40% followed by 'A' and 'B' positive that is, 22 and 20% respectively, 'A' negative and 'O' negative 4% each, however, no cases was seen in 'B' negative and 'AB' negative (Figure 1). In *P. falciparum*, maximum number of malaria were seen in blood group 'O' positive and 'A' positive that is, 40% each followed by 'B' and 'AB' positive, that is, 10% each, however no case was seen in 'A' negative, 'B' negative, 'AB' negative and 'O' negative (Figure 2).

Table 3. Showing correlation of prevalence of malaria in different blood group.

Workers	A (%)	B (%)	O (%)	AB (%)
Our study	16.08	21.95	25.86	13.04
Gayatri et al. (2013)	16.09	40.9	34.16	8.78
Deepa et al. (2011)	22	42	35	1
FD Olajevwo et al. (2013)	34.6	23.1	38.4	3.9
Sule Hussain et al. (2014)	32.3	35.3	17.7	24.2
Sing et al. (1995)	14.3	11.1	13.9	0
Tewodros et al. (2011)	23.5	21.9	51.3	3.3

**Figure 1.** Showing distribution of blood groups in *P. vivax* infection.

In mixed species, maximum number of malaria were seen in blood group 'O' positive, that 40% followed by 'A' positive 'B' and 'AB' positive that is, 20% each, however no case was seen in 'A' negative, 'B' negative, 'AB' negative and 'O' negative (Figure 3). Malaria cases were found mostly in O positive blood group patients (25.86%), followed by B positive (21.95%), A positive (16.08%) and AB positive (13.04%) (Table 2). As regards correlation, there are some differences from other reports. High incidence of malaria in O positive blood group (25.86%) was found in the study of Olajevwo et al. (2013), Tewodros et al. (2011) also reported higher incidence in O blood group 38.4 and 51.3% respectively. Gayatri et al. (2013) and Deepa et al. (2011), however reported higher incidence in B blood group 40.97 and 42% respectively. Less incidence of malaria was reported in blood group A and AB by most workers. Similarly, less incidence was found in A Rh negative and O Rh negative blood group

(12.5% each). Malaria was not found in B Rh negative and AB Rh negative. However, this number is small for any conclusion. Blood groups 'A' positive, 'B' positive, 'AB' positive, 'O' positive was statistically significant. (Table 4).

Regarding blood group and species distribution, it was observed that maximum incidence of *P. vivax*, *P. falciparum* and mixed species was in blood group O positive 40, 40 and 40% respectively (Table 5). Possible explanation for higher prevalence of malaria infection by earlier mentioned species, could be that there are no blood group antigens on the surface of O group red cells, and hence more number of receptors and chances of attachment of malarial parasites, where in blood group A, B and AB, the red cells are covered with respective blood group antigens and there is less number of receptor for malarial parasites and less chances for attachment of malaria parasite to these red cells.

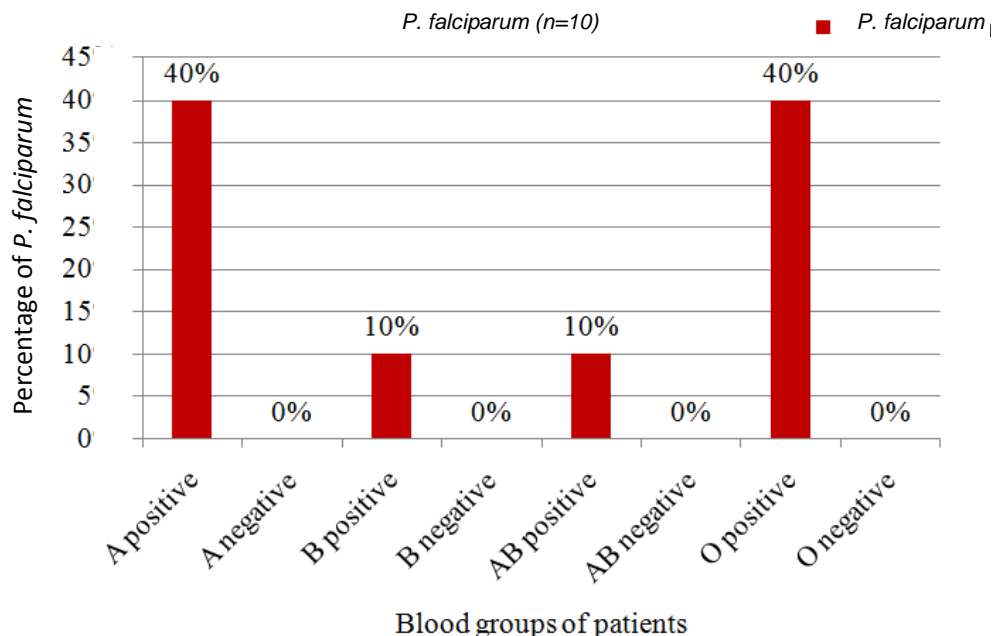


Figure 2. Showing distribution of blood groups in *P. falciparum* infection.

Table 4. Showing significant difference between Rh positive and Rh negative blood groups.

Blood groups	Negative	Positive	P value
A Positive	56	9	< 0.05*
A Negative	8	1	> 0.05
B Positive	41	9	< 0.05*
B Negative	4	0	> 0.05
AB Positive	23	3	< 0.05*
AB Negative	2	0	> 0.05
O Positive	58	15	< 0.05*
O Negative	8	1	< 0.05*

*Statistically significant (P value < 0.05).

Table 5. Showing blood group and overall malaria prevalence.

Parameters	Rh Positive				Rh Negative			
	A (%)	B (%)	AB (%)	O (%)	A (%)	B (%)	AB (%)	O (%)
<i>P. vivax</i> (n=25)	24	20	8	40	4	0	0	4
<i>P. falciparum</i> (n=10)	40	10	10	40	0	0	0	0
Mixed species (n=5)	20	20	20	40	0	0	0	0

Conclusion

This study, found out that ABO and Rh blood groups of human beings may show differences in susceptibility to malarial infection, the total blood samples were 200 included in this study, out of which 40 samples were

positive for malaria and 160 was negative. The A, B, O and Rh blood group was done using Eryscreen® of both positive as well as negative malaria cases. Maximum numbers of malaria positive cases were seen in blood group 'O' positive followed by 'A' 'B' positive 'A' and 'AB' positive. This study suggests that the person having

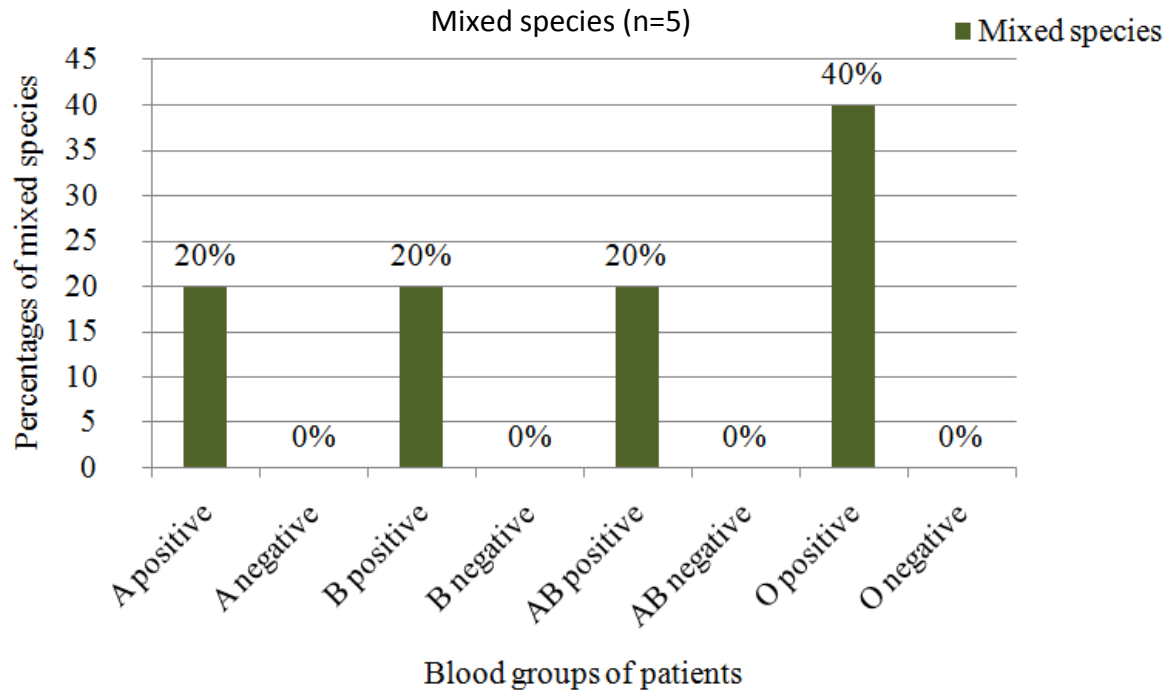


Figure 3. Showing distribution of blood groups in mixed infection.

blood group 'O' are more prone to malarial infection in endemic areas.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Prevalence of bovine fasciolosis in and around Bahir Dar, North West Ethiopia

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A cross sectional study was conducted from November, 2013 to April, 2014 to determine the prevalence and associated risk factors of bovine fasciolosis in and around Bahir Dar. A total of 384 fecal samples were examined by sedimentation and 124 (32.3%) were found positive for bovine fasciolosis. Risk factors such as site, sex, age, body condition score and breed were taken into consideration. Prevalence differed significantly between study sites ($P < 0.05$): 54% in Sebatamit, 41.2% in Addisalem, 19.2% in Zenzelma and 16%, in Yibab. Similarly, 48.4 and 30.9% prevalence were recorded for cross and local cattle breeds respectively. There was statistical significance difference between prevalences recorded in cross (48.4%) and local breeds (30.9%; $P < 0.05$). Prevalence based on sex showed 35.7% in male and 29.2% in female individuals, in age 29.2% young and 33.6% adult, and in body condition score 29.9% good, 31.2%, medium and 34.2% were recorded. There was higher prevalence in male than female, adult than young and poor body condition than good and medium body condition score. However, no statistical significance ($P > 0.05$) was observed for these three variables (sex, age and body condition score). This study showed that fasciolosis is prevalent in the study area, and this signifies the need for intervention through awareness creation on the preventive strategies among farmers and veterinarians.

Key words: Bahir Dar, bovine, fasciola, prevalence.

INTRODUCTION

Agriculture is the main source of Ethiopian economy. More than 38 million cattle and 39 million small ruminants, 8.6 million equine, 1 million camels, and 55.4 million chickens, consists major portion of livestock resource in Ethiopia (Zelalem and Fletcher, 1993; Asfaw, 1997; Ayele et al., 2003). The diverse ecology in Ethiopia makes Ethiopia home to a large population of different domestic and wild animals with considerable contribution to the national economy (Desalegn and Yermineh, 2004). Ethiopia's livestock productivity,

despite its huge livestock population size, remains marginal due to prevalent diseases, malnutrition, and management problems. Parasitism represents a major problem/obstacle to the development of this sub-sector (Malone and Yilma, 1998). Bovine fasciolosis is an economically important parasitic disease of cattle. The economic losses due to fasciolosis throughout the world (including Ethiopia) are enormous and these losses are associated with mortality, morbidity, reduced growth rate, condemnation of fluky liver, and increased susceptibility

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to secondary infections and expense of control measures and treatment (Bowman et al., 2003).

Fasciolosis is the diseases of ruminants with zoonotic potential. Humans are infected by eating aquatic plants containing encysted metacercariae or drinking contaminated water (Andrew, 1999; Mark and Dalton, 2009; Smith and Sherman, 2009). The two species most commonly implicated as the etiological agent of fasciolosis are *Fasciola hepatica* and *Fasciola gigantica*. *F. hepatica* has a worldwide distribution but is predominant in temperate zones, while *F. gigantica* is also found in most continents primarily in tropical (Andrew, 1999). The definitive hosts for *Fasciola* species are mammals, particularly cattle and sheep. The geographic distribution of parasitic trematode species is dependent on the distribution of suitable species of snails which act as intermediate hosts. The genus *Lymnaea* in general and *Limnaea truncatula* in particular, are the most common intermediate hosts for *F. hepatica*. This species of snail was reported to have a worldwide distribution. The intermediate host for *F. gigantica* in Africa is *Lymnaea natalensis* (Urquhart et al., 1996).

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 by several studies (Wondwosen, 1990; Mulugeta et al., 1993; Mezgebu, 1995). The prevalence of bovine fasciolosis has shown to range from 11.5 to 87% (Malone and Yilma, 1998). Fasciolosis is the priority diseases in the highland and low land areas of Amhara region (Solomon and Abebe, 2007). *F. hepatica* was shown to be the most important fluke species in Ethiopian livestock with a geographic distribution ranging over three quarter of the nation except in the arid northeast and east of the country. The spatial distribution of *F. gigantica* was mainly localized in the western humid zone of the country that encompasses approximately one fourth of the country (Malone and Yilma, 1998). The disease is found in vast water lodged and marshy grazing field condition. These provide suitable habitats year round for the snail intermediate hosts (Solomon and Abebe, 2007). Even if there were some study and reports about bovine fasciolosis in and around Bahir Dar; there was no any documents (study) about bovine fasciolosis in Sebatamit Addisalem, Yibab and Zenzelma sites specifically (sites of present study). Therefore, the objectives of this work is to determine the prevalence of bovine Fasciolosis in the study area (selected sites) in and around Bahir Dar and to assess the epidemiological risk factors that might contribute for fasciolosis, and to generate valuable base line information for further studies that will be conducted in the area

MATERIALS AND METHODS

Study area

A cross sectional study was conducted from November, 2013 to

April, 2014 in and around Bahir Dar. Bahir Dar is the capital city of Amhara regional state, which is found 565 km away from Addis Abeba, northwest of Ethiopia. The altitude of the area is 1500 to 2600 m.a.s.l. Bahir Dar is located between 12° 29'N latitude and 37°29'E longitude with an average annual rain fall ranging from 1200 to 1600 mm, annual temperature ranging from 8 to 31°C. About 70% of the land is featured by plain plateaus and covered by various bush formation, low woods mainly ever green lands some semi-humid highland vegetation planted with major agricultural products like teff, wheat, maize and pulse crops (CSA, 2009). Bahir Dar Zuria Woreda has a livestock population of 152,772 animals (121,470 cattle, 14,322 sheep, 9,141 goats and 7,839 equines 36,666 poultry). The biggest lake and river in Ethiopia, Tana and Blue Nile (Abay) are found in this area. The sites were selected by using simple random sampling around Bahir Dar.

Study animals

The study animals were cattle that were found in the selected sites in and around Bahir Dar (Addisalem, Sebatamit, Zenzelma and Yibab kebeles). The cattle include both local and cross between local and Holstein fresian breeds. A total of 384 cattle of all age groups and both sexes are randomly selected in the fields in different origins. The average cattle age determine by dentition as young and adult (Cringoli et al., 2002), and body condition score was recorded (Nicholson and Butterwort, 1986).

Study design and period

A cross-sectional study design was used to determine the prevalence of bovine fasciolosis, and its associated risk factors in and around Bahir Dar town from November, 2013 up to April, 2014. Breed sites (origin), age, sex and body conditions were considered as the risk factors for the occurrence of fasciolosis.

Sample size determination

The desired sample size for the study was calculated using the formula giving by Thrusfield (2005) with 95% confidence interval, 5% absolute precision and 50% expected prevalence as showed below:

$$N = \frac{1.96^2 \times P \exp (1 - P \exp)}{d^2}$$

Where

n = the required sample size,
P exp = expected prevalence,
D = absolute precision.

Therefore, based on the formula the total sample size calculated were 384. From the total of 384 sample, size 100, 85,100 and 99 cattle were selected at Sebatamit Addisalem Yibab and Zenzelma, respectively.

Sampling methodology and laboratory technique

Fecal (Coprological) examination

Fecal samples were recorded to data recording format. The age of the animals was recorded by interviewing owners and cross-checked using dental formula (Cringoli et al., 2002). The study sites were randomly selected. During sample collection: sex, body

Table 1. Prevalence of Bovine fasciolosis based on fecal examination.

Number of animal examined	Total number of positive prevalence	Percentage (%)
384	124	32.3

condition and breed, were also recorded. Coprological examination was conducted on fecal samples collected directly from the rectum of the animals into a universal bottle containing 10% formalin and transported to Bahir Dar Regional Veterinary Laboratory for examination. Sedimentation technique was used to detect the presence or absence of fluke eggs in the fecal sample collected, as described by Antonia et al. (2002). To differentiate between eggs of *Paramphistomum* species and *Fasciola* species, a drop of methylene blue solution was added to the sediment where eggs of *Fasciola* species show yellowish colouration while the eggs of *Paramphistomum* species stain by methylene blue (Hanson and Perry, 1994). The equipments and tools used for the study were beaker, strainer, measuring cylinder, mortar and pestle, test tube, test tube rack, microscope slide, cover slip and microscope. The procedure for the examination of fasciola egg is as follows: About 3 g of faeces is collected from the rectum of each selected cattle using sample bottle. The faecal sample should be crushed with mortar and pestle, and 40 to 50 ml of tap water should be added and mixed with stick and thereafter, the faecal suspension is then filtered through a tea strainer into a beaker, the filtered material should be pour into a test tube. After pouring into a test tube wait for 5 min and the supernatant fluid will discarded carefully using a pipette, transferred a small amount of the top of the layer of the sediment to a microscope slide and covered with cover slip, then examined under 40xs magnification power (Hendrix and Sirois, 2007).

Data analysis

All raw data generated from this study were entered into Microsoft office excel data base system. The findings were analyzed using statistical package for the social sciences (SPSS) version-17.0 computer program; and data were analyzed using Chi-square (χ^2) to determine the variation in infection, prevalence between sex, breeds, study sites, body condition and age groups. The total prevalence was calculated by dividing the number of disease positive animals by the total number of animals examined (Table 1). Statistical significance was set at $P < 0.05$ to determine whether there are significant differences between the parameters measured between the groups.

RESULTS

Prevalence of bovine fasciolosis

Of the total 384 fecal samples examined under microscope by sedimentation technique, 124 animals were infected by fasciolosis and an overall prevalence of 32.3% was recorded for fasciolosis in the study area (Table 1).

Prevalence of fasciolosis on study site basis

The prevalence of fasciolosis varied significantly among

the four areas (origins), and the highest prevalence of fasciolosis was observed in Sebatamit (54%), followed by Adissalem (41.2%), Zenzelma (19.2%) and Yibab 16 (16%) indicated in Table 2. Statistical analysis revealed that there was significant difference ($P < 0.05$) in infections between areas.

Sex differences in the prevalence of fasciolosis

Prevalence of fasciolosis was 35.7% from a total of 185, and 29.1% from a total of 199 in male and female animals respectively. No significant difference observed between sexes ($P > 0.05$) (Table 3).

Breed differences in the prevalence of fasciolosis

There was statically significance difference between the prevalence of bovine fasciolosis with different breed of animals ($p < 0.05$). Out of 353 local animals, the prevalence was 30.9% (109) which came from 31 cross animals. The recorded prevalence was 48.4% (15) (Table 4).

Age differences in the prevalence of fasciolosis

Prevalence of fasciolosis in bovine adult was higher than of the young. Out of 271 adults, the prevalence was 33.6% and out of 113 young the prevalence was 29.2%. There was no statically significance difference between prevalence of bovine fasciolosis within different ages of animals ($p > 0.05$) (Table 5).

Prevalence of fasciolosis and body condition score

Among the 384 animals, 161 were determined to be of poor Budd-Chiari syndrome (BCS), 197 medium BCS and 26 good BCS. The prevalence in these three groups was 62 (34.2%), 54 (31.5%) and 8(29.6%) respectively (Table 6).

DISCUSSION

Fasciolosis represents one of the most common liver parasites in domestic ruminants and humans, and is present throughout the world where the climatic conditions are suitable for snails (*Lymnaea* spp.), the

Table 2. Prevalence of bovine fasciolosis on sites/locality basis.

Site	No of animal examined	Positive	Prevalence (%)	χ^2	P-value
Sebatamit	100	54	54	44.532	0.000
Adissalem	85	35	41.2		
Yibab	100	16	16		
Elma	99	19	19.2		
Total	384	124	32.3	-	-

Table 3. Prevalence of bovine fasciolosis as compared with sex.

Sex	No of samples examined	Positive	Prevalence (%)	χ^2	P- value
Male	185	66	35.7	1.870	0.172
Female	199	58	29.1		
Total	384	124	32.3	-	-

Table 4. Prevalence of fasciolosis in cattle based on breed.

Breed	Sample size	Positive	Prevalence (%)	χ^2	p- value
Local	353	113	30.9	48.4	3.996
Cross	31	15	0.046		
Total	384	128	32.3	-	-

Table 5. Prevalence of bovine fasciolosis on age basis.

Age	No of examined animal	Positive	Prevalence (%)	χ	p- value
Young	113	33	29.2	0.698	0.403
Adult	271	91	33.6		
Total	384	124	32.3	-	-

Table 6. Prevalence of bovine fasciolosis on body condition basis.

Body condition	No of animal examined	Positive	Prevalence (%)	χ^2	p-value
Good	27	8	29.6	0.462	0.794
Medium	199	62	31.2		
Poor	158	54	34.2		
Total	384	124	32.3	-	-

intermediate hosts of *F. gigantica* and *F. hepatica* (Urquhart et al., 1996). The infection is considered a neglected disease according to the World Health Organization (WHO, 2012) and where animal cases are reported, human cases generally also exist. In general, fasciolosis was found prevalent in the study areas. This

will be a hindrance to the livestock production by causing remarkable direct or indirect losses in the study areas. The present study was designed to determine prevalence and assess risk factors associated with bovine fasciolosis by coprological examination. Based on coproscopical investigation, an overall prevalence of 32.3% bovine

fasciolosis was revealed, it is higher than the prevalence (21.22%) reported by Ayalew (2011) in and around Bahir Dar, and the prevalence of (24%) at Andassa Livestock Research Center (Asressa, 2011), and current prevalence was nearly similar to that of the prevalence (36.7%) studied by Fikremariam et al. (2009) in Bahir Dar but it was less than that of the findings at Woreta with prevalence of 41.41% by Biniam et al, 2012. This difference may be attributed to various factors such as the distribution of the study period and other factors such as availability of suitable snail habitat (Urquhart et al., 1996). This may also be associated with the sedimentation technique as the aggregated distribution of eggs in faeces makes it easy to miss eggs by taking just 3 g; eggs can be lost during sedimentation.

The prevalence of bovine fasciolosis was different in different districts (location). The higher prevalence of bovine fasciolosis was recorded at Sebatamite that is, 54%, this difference might be due to the difference in geographical location favorable to maintain the intermediate host and the disease. The prevalence of the disease in the four different sites of the study areas were 54, 41.2, 16 and 19.2% in Sebatamit, Addisalem, Yibab and Zenzelma respectively. This showed statistical difference in the prevalence of the disease between these sites ($P < 0.05$). However, higher prevalence in Sebatamit followed by Adissalesmm, due to its near location to the Abay River basin and Lack Tana which is permanently wet and water logged maintaining cyclic lifecycle of the parasites intermediates host, but the rest two origins were dry areas. The samples were taken at the same season of the year. In sex, the prevalence of the bovine fasciolosis in male and female animals was recorded as 35.7 and 29.1% (Table 3), respectively. Even if there were difference in prevalence, there was no significant difference ($P > 0.05$) between the two groups. Sex had no effect on the prevalence of the disease, because the grazing pasture land or management system was same and equally exposure to a similar contaminated pasture by both sex groups and traditionally animals are driven to pasture regardless of sex. Similar results that support the present finding were reported by Solomon and Abebe, 2007 and Fikirtemariam et al. (2009). This signifies that sex has no impact on the infection rate and both male and female animals were equally susceptible and exposed to the disease. Similar results that support the present finding were reported by Biniam et al. (2012).

In this study, the prevalence of fasciolosis in bovine showed higher in adult than young. Out of 271 adult, the prevalence was 33.6% and from 113 young the prevalence was 29.2%. No statically significance difference between prevalence of bovine fasciolosis within different ages of animals ($P > 0.05$).

In different parts of Ethiopia, different results indicating inverse correlation of prevalence and age of cattle were reported by Wondwossen (1990) and Rahmato (1999). As the age increased to the adult stage, the magnitude of

infection rate increased to a higher level. As the age of the animal increases, the possibility of being exposed to *Fasciola* increases and hence high prevalence of fasciolosis may be observed. In general, the younger the age the lower the prevalence and the older the age the higher the prevalence and this could be due the maternal immunity acquired by young animals through colostrums. This finding would not agree with the works of Solomon and Abebe (2007), and Yilma and Mesfin (2000) where the detections of *Fasciola* eggs were lower in the young group. This might be due to the fact that young and older (adults) age groups were grazing and watering in same area and had equal chance of being exposed to infection.

The infection rate of bovine fasciolosis on the basis of breed showed statistically significant difference ($P < 0.05$). Infection rate in cross breeds was (48.4%) higher than local-breed (30.9%), this could be due to differences, cross breeds are genetically susceptible than local breeds and they were high chance of being exposed to this diseases due to the high amount of feed as well as the farmers reared under traditional husbandry system, that is, local animals are resistance. The reason for high prevalence of fasciolosis and significantly difference ($P < 0.05$) in cross breed compared to local breed might be due to lower resistance of cross than local breed.

Body condition was a risk factor for bovine fasciolosis and prevalence was 34.2, 31.2 and 29.6% in poor, medium and good body condition score respectively. There was no significant variation among body condition scoring groups. The lower prevalence was observed in animals with good body condition (29.6%) and the higher was in animals with poor body condition (34.2%). This might be association (due to) with animals with poor body conditions were less resistance as a result of malnutrition or presence of other chronic diseases and environmental conditions that make minimum parasite loads to overcome the host immunity are made available. Similarly prevalence of other concurrent infection (parasitic or non parasites) might make the animal to have poor body condition. Their existence along with fasciolosis might have impact on body condition and body weight of the animals (Wassie, 1995).

CONCLUSION AND RECOMMENDATIONS

Bovine fasciolosis is a major problem in animal production, which causes decreases production, fertility, emaciation and disease and finally death. The result of this study indicates fasciolosis is 32.3% prevalent. The occurrence is closely associated with presence of suitable environmental conditions for the development of snails. The study area is suitable for the survival of the snail which worsened the situation for the future. The high prevalence reported in this study has clearly indicated lack of strategic control measures against the disease, and due to the risk of water lodgment from Abay rivers and Lake Tana which increased irrigated land

masses and ponds at grazing areas, and the tendency of farmers to graze their animals in these areas because of feed scarcity. In general, this study indicated that fasciolosis is an important infection to livestock development in the study area. Based on the conclusion, the following recommendations are forwarded:

1. Awareness creation on the preventive strategies such as drainage of marshy areas, clearing of aquatic vegetations and seasonal strategic deworming of animals should be practiced.
2. Draining or fencing of marshy areas, utilization of swampy areas for crop production, to protect the animals from infection during grazing.
3. Strategic application of fluckicide and avoiding animals grazing from marshy land plays considerable success for the control of fasciolosis in these study areas
4. Training need to be organized for farmers with economical significance and control methods of this disease in the study area.
5. Detail epidemiological study should be carried out on biology and ecology of the intermediate host so as to develop a substantiable planning and implementation on the control strategies of the disease.
6. Expansion of animal health care delivery in the area including drug supply, prophylactic and other disease control strategies.

Conflict of Interest

The authors declared that they have no conflicts of interest.

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