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In Sennar; out of the 218 donkeys examined for parasitic infestation, (193) donkeys were positive and the prevalence was 88.53 %. (El Dirdiri *et al.*, 1986). Seri *et al.*, (2004) examined 1200 donkeys in Khartoum state and reported prevalence with nematodes infection in donkeys of (70.1%). In Nyala Sawsan *et al.*, (2008), examined (810) donkeys, among which 302 were found to be positive for helminth infestation and the prevalence rate was 37.48 %.

Cope with the parasitism and deleterious consequences, various effective parasiticide have been developed in the past relying on molecules such as macrocyclic lactones' (Ivermectin and Moxidectin), praziquantel, for internal parasites and Benzimidazole derivative (Albendazole, Fenbendazole, and oxybendazole), for internal parasites, introduced as a veterinary endectocide (Egerton *et al.*,1980).

Ivermectin (IVM) has proven to be a potent broad spectrum anthelmintic against gastrointestinal nematodes (Klei *et al.*,2001). Abamectin (avermectin B1) is a natural fermentation product of *Streptomyces avermitilis* that differs from ivermectin (a synthetic derivative of Abamectin) only in the bond between carbons 22 and 23. Abamectin has a single bond while ivermectin has a double bond and additional hydrogen on C-22 and C-23 (Lankas and Gordon, 1989).

On the other hand praziquantel (PRZ) was shown to be highly effective against Trematodes (*Flukes*) and Cestodes, (*Tapeworm*), featuring an antiparasitic spectrum somewhat different from that of (IVM) Slocombe *et al.*, 2007). As a matter of principle, the drugs that associate two or more molecules with different activity profiles a proved to be a potent broad spectrum anthelmintic against gastro intestinal nematodes (Klei *et al.*, 2001).

The health and welfare of the donkey is of importance to those people who depend on it. Studies of parasites in working donkeys have uncovered adversity of helminthes species (Getachew *et al.*; 2008).

Egg reappearance period (ERP) can be defined as the time from anthelmintic use till parasite eggs can again be detected in the faeces. It was originally introduced as a tool to help designing treatment regimens and specifically determining the intervals between different treatments in the intervals dose regimens, but is now used as surveillance tool for indicating developing levels of resistance. The egg reappearance period (ERP) is defined as: the period after anthelmintic treatment in which mean egg count do not exceed 100 EPG (Boersema *et al.*, 1995). Some studies have defined it as the week of the first positive egg count post treatment (Dudeney *et al.*; 2008). Others have used a fixed threshold of the mean egg count, such as 100 or 200 EPG (Mercier *et al.*, 2001). A third definition of ERP is to use the

faecal egg count reduction test (FECT) to calculate weekly efficacies and then use a predetermined cut-off value for defining the ERP (Boersema *et al.*,1995).

In this study helminths infestation in donkeys was investigated in El fashir city North Darfur State, and study the therapeutic efficacy, persistent effect and treatment intervals of two avermectin compounds.

The objectives of this study are to:-

1. Determine the prevalence of common GIT nematode parasites infectings donkeys present in Elfashir, North Darfur State.
2. Evaluate the therapeutic efficacy of abamectin and ivermectin in donkeys naturally infected with gastrointestinal helminths.
3. Report on the persistent effect and treatment intervals of the drugs under investigation.
4. Determine the change resulting from treatment on the blood biochemical profile.

Chapter one

Literature review

Macrocyclic lactones .1

Macrocyclic lactones (MLS) are compounds that have activity against both internal and external parasites, especially nematodes and arthropods, however have no activity against *cestodes*, *trematodes* or *protozoa*. Macrocyclic lactones are fermentation products from soil dwelling fungi of the genus *Streptomyces avermitilis*. In addition to having broad spectrum activity they are effective at very low concentrations. Macrocyclic lactones comprise two major groups, the avermectins and milbemycins (Adams, 2001).

1.1 Avermectins

Avermectins and Milbemycins are closely related 16-member macrocyclic lactones)Burg *et al.*,1979(. It was the unique combination killing of endo-and ectoparasites that gave rise to the embracing name endectocide by which the MLs are recognized (Shoop *et al.*, 1995).

Information on the antiparasitic properties of the different ML compounds in all animal species has been thoroughly reviewed (Shoop *et al.*, 1995; Mckellar and Benchaoui, 1996). Avermectins are produced as a mixture of different components from fermentation of *Streptomyces avermitilis*. The highest anthelmintic potency is held by the B1 homologs. The avermectins family includes a series of natural and semisynthetic molecules, such as abamectin, ivermectin, doramectin, eprinomectin and selamectin (Vercruysse and Rew, 2002).

At Present avermectins are the active compounds of some insecticidal and nematocidal products used in agricultural and the most used agents in veterinary medicine for several years in prevention of parasitic diseases

(Float *et al.*, 2005). The major difference between avermectins and Milbemycins is disaccharide group attached at the C-13 of avermectin whereas that position is unsubstituted in milbemycins. The avermectins are produced as a mixture of eight different components from fermentation of *Streptomyces avermitilis* (Campbell *et al.*, 1983). These compounds are denoted as A1a, A1b, A2a, A2b, B1a, B1b, B2a, B2b, the 1 component have double bond between the C-22 and C-23 position, whereas 2 component have a single bond with hydroxyl group at C-23 position the component have a secondary butryl substituent at the C-25 position, while the b component have an isopropyl substituent at the C-25 position (Shoop *et al.*, 1995). A catalytic region selection hydrogenation of the naturally occurring avermectin B1, on the C-22 – C-23 double bond yields a C-22-C-23 dihydro derivative ivermectin.

1.2 Abamectin

Abamectin (ABM) is a macrocyclic lactone product derived from the soil microorganism *Streptomyces avermitilis*, It is a mixture of avermectins containing about 80% avermectin B1a and 20% avermectin B1b (Burg *et al.*, 1979; Fisher and Mrozik, 1989). These two components, B1a and B1b, have similar biological and toxicological properties (Lankas and Gordon, 1989). They are highly lipophilic substances and dissolve in most organic solvents, but are poorly soluble in water (Roth *et al.*, 1993). Although pesticides like ABM may be valuable in agriculture, it may be highly toxic to mammals (Moline *et al.*, 2000).

Abamectin is the naturally occurring avermectin approved for animal use, and the starting material for the production of ivermectin. In fact, ivermectin, is the first marketed endectocide molecule, is a semisynthetic derivative of the avermectin family. Ivermectin is the generic name given to

a mixture of two chemically modified avermectins containing at least 80% of 22-23-dihydroavermectin B1a and less than 20% of 22-23-dihydroavermectin B1b (Fisher and Mrozik, 1989). The relative popularity of the avermectin amongst the farmers and Veterinarians can be related to spectrum of activity, convenience and wide margin of safety to the targeted animals. Abamectin was widely employed to control insects and mites of a wide range of agricultural products such as fruit, and vegetable (Lankas and Gordon, 1989). Additionally as a safe chemical in mammals, abamectin has been used as an anthelmintic agent in both animals and humans (Kaplan *et al.*, 1994).

Abamectin oral formulation was markedly effective against ivermectin resistant strain of *T. colubriformis* in both faecal egg count and worm egg count reduction. Similar result with abamectin oral formulation were achieved against ivermectin resistant strain of *T. circumcincta* (Leathwick *et al.*; 2000), the higher efficacy of oral formulation of abamectin against ivermectin resistant strain of *T. colubriformis* than avermectin oral formulation, however abamectin injection formulation did not significantly reduce faecal egg and worm count of ivermectin resistant of *T. colubriformis*. Very little is known about the possible effects of abamectin on earth worm populations (Sun *et al.*, 2005). It should be noted that topical abamectin is approved for use in bovine species only in the EU and Australia, but not approved for veterinary use in the US. Because of differences in dermal anatomy and physiology between animal species, it is difficult to predict dermal absorption and disposition and residue depletion in other livestock species (Baynes *et al.*, 2000). Studies concerned with the effect of abamectin against ticks are scarce. The only reports available are those using pour-on preparation against *Rhipicephalus appendiculatus* and

Rhipicephalus decoloratus (Van Der Merwe *et al.*, 2005) and subcutaneous injection against *Boophilus microplus* (Pereira 2009).

1.2.1 Abamectin Identity

1.2.1.1 Molecular weight

Avermectin B1a :873.11

Avermectin B1b : 859.8

1.2.1.2 Chemical name

Component B1a R=C₂H₅:5-O-demethylavermectin A1a

Component B1a R=CH₃:5-O-demethy-25-de(1-methylpropyl)-25-(1-methyl) avermectin A1a

1.2.1.3 Molecular formula

C₄₈H₇₂O₁₄ (avermectin B_{1a}) + C₄₇H₇₀O₁₄ (avermectin B_{1b}).

1.2.1.4 Abamectin Structure

1.2.2 Abamectin mode of action

Macrocyclic lactones (MLs) are broad spectrum anthelmintics used to control nematode parasites of animals and humans. Macrocyclic lactones stimulate the release and binding of (GABA) at the nerve ending. This in turn opened GABA gated chloride channel and It is now known that macrocyclic lactones bind selectively and with high affinity to glutamate gated chloride ion channels in parasite nerve and muscle cell.

Macrocyclic lactones may potentiate GABA gated sites at high dosages. About 50% of the effect of macrocyclic lactones can be reversed with picrotoxin, GABA antagonist at the chloride channel. Macrocyclic lactones also interfere with reproduction of nematodes and arthropods parasites but the mechanisms of action are poorly understood. They may reduce

Figure 1.1: Abamection Structure
oviposition in ticks and abnormal egg formation in nematodes of
ruminants and sterility of both male and female filarial nematodes (Adams,

2001). Their mode of action includes strong chloride influx into nerve cells, which results in disruption of nerve impulses, blocks the channel causing nerve hyperexcitation and decreases nerve transmission. They are potent agonists at the GABA_A (gamma amino butyric acid) receptor but they also interact with GluCl (glutamate-gated chloride) channels in the nervous system of parasites (e.g., nematode). Visible activity, such as feeding and egg laying in parasites, stops shortly after exposure, though death may not occur for several days (Martin *et al.*, 2002).

Studies on the transport kinetics of anthelmintics when applied to parasitic nematodes in vitro suggested that trans-cuticular absorption may be a common mode of entry for anti-nematodal drugs it was suggested that the drug may enter the nematode body by crossing the cuticle or by entering openings other than the mouth (Ho *et al.*, 1994). The mode of action of the AVM on nematodes is much known, but a little is known about the means by which these drugs enter the nematode body and thus gain access to the relevant receptor (Geary *et al.*, 1993; Smith and Campbell, (1996).

1.2.3 Abamectin therapeutic efficacy

According to the world Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations a highly effective anthelmintic should have efficacy of more than 98% (Wood *et al.*, 1995). In trials ABM was used subcutaneously at dose 200µg/kg against adult and-fourth stage larvae (L4) of nematodes parasites in cattle including inhibited fourth stage larvae (L4) of *Ostertagi ostertagi* the efficacy values of abamectin was more than 99% (Benz and Cox, 1989). Williams *et al.*, (1992) reported the anthelmintic efficacy of abamectin (avermectin B1) was evaluated against gastrointestinal nematodes including *Ostertagi Ostertagi* inhibited larvae and lung worm the study showed 99% efficacy, removing

all primary stage of *Ostertagi ostertagi* inhibited larvae (L4), the lower efficacy was 93.8% against *D. viviparus*, in another study the endectocide activity of new long acting formulation containing 2.25 ivermectin +1.25 abamectin was explained in cattle in Brazil the anthelmintic efficacy of the avermectin against *Haemonchus placei*, *Cooperia spatulata* and *Cooperia punctata* was 89.64% , 98.84 and 97.69 %, respectively, (Borges *et al.*, 2007). The efficacy of abamectin was found to be similar to that of ivermectin. It effectively controlled infections with *H. placei*, *Ostertagia*, *Cooperia*, and *Oesophagostomum radiatum* at least 7 days after treatment in cattle, and *D. viviparus* up to at least 14 days after treatment (Barth,1983). Also another study was conducted in sheep, against Ivermectin resistant strain of *Trichostrongylus colubriformis* using abamectin oral and injectable at the dose rate of 0.2mg/kg body weight, abamectin oral and injectable and ivermectin were reduced faecal egg counts 66% ,98% and 76% respectively 10 days post treatment period (AlKa *et al.*, 2004).

Up to the present abamectin injection is registered in Australia, New Zealand, Brazil, Argentina, it is given subcutaneously at the dose rate of 200 µg/kg against adult and fourth stage larvae of nematode parasites in cattle including inhibited fourth stage larvae of *Ostertagia ostertagia*. The efficacy of abamectin was consistently greater than 99% (Benz and Cox, 1989).

Use of abamectin in field trial in Australia has been reported (Tahir *et al.*, 1986) and it was shown to be highly effective against an oxfendazole resistant strain of *T. axei* in cattle (Eagleson and Bowie 1986). On the other hand abamectin is more active against nematodes (Egerton *et al.*, 1979) and slightly less efficient against arthropods (Shoop and Soll, 2002), when compared to ivermectin. Abamectin in dosage of 200µg/kg was established

by the minimum lethal concentration for the species *Linognathus vituli* and *R. microplus*. Thus the is used to eliminate ivermectin dosage eliminate parasites of gastrointestinal nematodes, while abamectin is used for ectoparasites. This demonstrates the difference in antiparasitic activity of these two molecules.

1.2.4 Abamectin persistency effect

Macrocyclic lactones (ML) are known for their persistent anthelmintic effect against gastrointestinal nematodes. The major objective in strategic parasite control is to reduce the level of pasture contamination through treating animals to kill and to decrease eggs shedding from infected animals. In Zambia, Meeus *et al.*(1997), conducted a study using abamectin (0.2mg/kg) in cattle grazing naturally infected pasture and the animals were treated on day zero. The main genera of parasite found were *Cooperia* and *Heamonchus* in eggs examined pre treatment, the abamectin persistent effect was extended to day 48 post treatment and the cut point was (100 epg).

1.2.5 Abamectin toxicity

Some people committed suicide by ingesting abamectin and caused death in Taiwan (Chung *et al.*, 1999). So far, the cause of the patient's death is still unknown. Intoxication of abamectin may affect the function of hepatocytes although the permanent liver damage is usually not revealed immediately. A previous study has shown that abamectin causes serum aspartate aminotransferase (AST) elevation (Lowenstein *et al.*, 1996). Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt, 1982). AST is used to detect liver damage (Klaassen and Eaton, 1991). Environmental exposure to these agents may

cause serious health risks including fertility and reproductive function. The Previous reports have indicated a strong link between male infertility and exposure to more than 50 pesticides (Manfo *et al.*, 2010; Tiwari *et al.*, 2011). The widespread use of ABM has stimulated research into the possible existence of effects related with their reproductive toxic activity. Despite the large amounts of research on the various toxic effects of ABM (Sun *et al.*, 2005), there are limited number of studies evaluating its effect on fertility and reproduction (Xu *et al.*, 2005). Furthermore, the mechanisms underlying It's possible harmful effects on male fertility are not known.

1.3. Ivermectin

Ivermectin (IVM) was the first Macrocyclic Lactone anthelmintic introduced as a veterinary antiparasitic agent in France in 1981. Ivermectin is marketed as mixture of 22, 23 dihydro B1a (>80%) and 22,23 dihydroB1b(<20%) (Fisher and Mrozik, 1989). Extremely small quantities (less than 1mg/kg) of ivermectin are sufficient for anthelmintic activity by either oral or parenteral route of administration. Confirmation tests with ivermectin have indicated a wide range of efficacy against nematodes and many arthropods (Campbel and Benz 1984). The parasites that are eliminated by ivermectin included all major GIT and lung nematodes and a certain ectoparasites of cattle, sheep, horses and swine (Adams, 2001).

1.3.1 Efficacy of Ivermectin against equine nematodes

According to Seri *et al.*, (2005), the efficacy of ivermectin injectable formulation at dose of 200µg/kg in donkeys was reveled 100%. Small *strongyles* Known as *Cyathostomes* occur in the large intestine of the equides all over the world (Soulsby, 1982). Burrows and his colleagues (1985) used faecal examination to demonstrate the efficacy of Ivermectin paste formulation at 200µg/kg against benzimidazole-resistant small

Strongylus, the egg counts remained zero at least 3 weeks in horses. Also 200-300 µg/kg were effective against *Cyathostomes* in 99% of cases and the efficacy was 86% - 97% against immature stages of *Cyathostomes* following 200-300µg/kg dose rate respectively (DiPietro *et al*; 1982). There are three species of *Strongylus* namely *equinus*, *S. vulgaris* and *S. edentatus* found in the large intestine including the caecum of equines (Soulsby, 1982). Generally *strongylidae* worms are not severe pathogens, unless they occur in large numbers when their mouth parts cause extensive damage when sucking in the mucous membrane causing ulcers.

Larvae, however, in the characteristic nodules, produced damage, which resulted in bacterial invasion and consequently ulcers. When the larvae leave the nodules extensive bleeding occurs (Hall, 1985).

Fourth-stage larvae of *S. vulgaris* penetrate the intima of the sub-mucosal arterioles and migrate in the vessels towards the cranial mesenteric artery where reside for 14 days after infection causing thrombi and later aneurysms (Soulsby, 1982).

The oral paste and injectable formulations of Ivermectin at 200 µg/kg was 100% effective against *Stongylus vulgaris* in 8-week-old ponies, , as revealed by necropsy 5 weeks after treatment (Klei *et al.*, 1984). Also intramuscular injectable formulation at dose rate 200µ g/kg body weight showed that the efficacy of Ivermectin against arterial stages of *Strongylus vulgaris* was only 69, 23% (Seri *et al* ; 2005). *Strongloides westeri* occurs in the small intestine, mainly in the duodenum and jejunum which causes irritation, resulting in diarrhea, especially in young foals. 200µ g/kg of Ivermectin was effective against *S. westeri* in foals using paste formulation (Ryan and Best 1985). *Trichostrongylus axei*, a small worm (3-8mm) found in the stomach and duodenum of horses. *T. axie* caused chronic catarrhal

gastritis (Hall, 1985). *H. musca*, *H. microstoma* and *D. megastoma*, occur in the stomach of equidae. The largest one is the *H. microstom*. The first 2 *Draschia spp* and *Habronema spp* are free in the host's stomach; *D. megastoma* lives in nodules in the stomach wall. Diagnosis of *Habronemiasis* is often impossible because the larvae can seldom be found in the faeces (Hall, 1985). In horse, Herd and Donham (1983), reported that 1-2 intramuscular doses of ivermectin at 200 µg/kg were highly effective against *Draschia spp* and *Habronema spp.*, and DiPietro (1982) reported 100% efficacy.

The larvae of several species of the *Gastrophilus spp* genus are parasites of equidae and are known as bots flies. The third- stage larvae causes inflammation and ulceration of the mucous membrane of the stomach and duodenum in addition to blood sucking (Soulsby, 1982; Hall, 1985).

Ivermectin at rate of dose rate of 200-300µ g/kg given intramuscularly to horses had 99% efficacy against larvae of *Gasterophilus spp*, the few larvae in the treated horses were located in the large intestine contents and were not attached to the intestinal epithelium (DiPietro; 1982).

Parascaris equorum is found in the small intestine of horses. Both infection and migration is through the blood stream via the right ventricle of the heart to the lungs (Hall, 1985).

Ivermectin at 200 µg/kg given orally as paste formulation, had totally eliminated the passage of *P. equorum* in naturally infected horses (Cobra *et al.*, 1986). Ivermectin is also active against immature stages of *P. equorum*, in ponies, larval burdens were determined at necropsy 2 weeks following treatment with the paste at 200 µg/kg. The results obtained showed 100% reduction in lung larvae burden (French *et al.*, 1988) and e 100% reduction in intestinal larvae burden (DiPietro *et al.*, 1987).

Both the paste and liquid formulations of ivermectin have been shown to be highly active against both the early (L3) tissue phase of *P. equorum* and against the later intestinal (L4) phase of *P. equorum*. The efficacy was discussed by Boraski (1987).

1.3.2 Resistance to ivermectin

Unfortunately, resistance has developed to ivermectin and the first indication of ivermectin resistance was reported from South Africa (Carmichael *et al.*, 1987), against *Haemonchus contortus* isolated from sheep. Subsequently, there has been worldwide incidence of ivermectin resistance in various species of nematodes (Gopal *et al.*, 1999). This burgeoning problem of ivermectin resistance in sheep parasites has drawn the attention of veterinarians and farmers to opt for alternative drugs and control strategies.

1. 4 Prevalence of gastrointestinal nematodes in equines

In Sudan (Ibrahim *et al.*, 2011), examined 187 faecal samples of working donkeys in Khartoum State, 119 were found positive for gastrointestinal parasites. The parasites encountered were *Strongyles* 67.38%, *Strongyloides westeri* 55.46%, *Parascaris equorum* 20.17%, *Anoplocephala perfoliata* 5.88%. In Nyala (Ahmed, 2008) examined 92 donkeys 90 donkeys were found with infected by one or more internal parasites with an overall prevalence of 97.83%. Sawsan *et al.*, (2008), conducted a similar survey in Nyala the overall prevalence of nematode parasite in horses and donkeys of 29.29%, while the nematode prevalence in horses and donkeys were 15.73% and 37.48% respectively. Seri *et al.*, (2004), reported that, the prevalence of nematode was 70.1% in Khartoum reported state *Strongylus* 35.8%, *Cyathostomes*.36.7%, *Parascaris equorum* ,10.7% *Trichostrongylus axei*, 12%, *Strongyloides westeri* 3.4%.

Eisa *et al.*, (1979) , reported only two helminthes parasits (*Oxyuris equi* , *Seteria equina*), were recorded in equine in Darfur region. Kheir and Kheir (1981) conducted a field survey in South Darfur State, and stated that the overall prevalence was 56.2% and the overall prevalence of infection with nematode parasites was found 58% in town animals and 22% in nomadic areas. Five nematode genera were encountered in donkeys in the same area *Strongylus spp* (43.6%), *Oxyuris spp* (10.5%), *Strongloides* (3.9%), *Parascaris equorum* (4.4%) and *Trichuris* (0.9%). The prevalence of gastrointestinal

nematodes in horses in Bahr Al Arab area was (18.5%). In Sennar State El Dirdiri *et al.*, (1986), examined 218 donkeys for parasitic infestation. He reported that 193 donkeys were positive and the prevalence was (88.53%), the prevalence of *Strongylus spp*, was 100% and *Oxyuris spp* 0.5%, both highly and moderately infested animals and slightly infected ones were 36.27 and 27.46% respectively.

Another study conducted by Mukhwana, (1994), reported that the prevalence of *Strongylus spp* was 57.6%, *Cyathostomes spp* was 15.4% and *Parascaris equorum* was 20.7%. In Morocco the prevalence of different gastrointestinal nematodes species reported were as follows: *Cyathostomes* 52%, *Parascaris equorum* 37% and 93.5%, *Trichuris spp* (Abdelkarim 1991). In Chad , Garber (1970), reported that the prevalence of both *Strongylus spp* and *Cyathostomes spp* were 89% ,72% for *Parascaris equorum* and 6% for *Strongyloides westera*. Ayele *et al.* (2006) examined 338 samples and found positive helminthes eggs for *Strongylus spp*100%, *Parascaris equorum* 50% and *Oxyuris equi* 3% in Ethiopia. Nationwide survey in Sweden showed that *Strongylus* infection was highly prevalent in

horses 78% of 1183 examined horses on 110 farms were found to shed nematode eggs (Lind, 2005).

1.5 Blood biochemistry changes in donkeys treated with Doramectin

In Khartoum State Seri *et al.*, (2006) conducted a study to evaluate the subacute toxicity of doramectin injectable formulation in healthy donkeys. The trial covered four groups of animals each of five; three treated groups received doramectin injection intramuscularly at 100, 200 and 300 µg/kg body weight for seven continuous days. The results obtained from treated groups were compared to control group, only urea showed significant increased ($P < 0.05$) both in treated and control groups, urea showed significant decrease in animals that received 100 µg/kg body weight, the result obtained indicated that Doramectin has no toxicity and safe to be used in donkeys. The same author investigated the effect of doramectin injectable formulation on some blood biochemical constituents of the donkeys. Selected blood biochemical constituents were examined in 2 groups of donkeys naturally infected with *Onchocerca raillieti* before and after treated with doramectin injectable formulation. The animals received doramectin injection at the dose of 200 µg kg⁻¹ body weight either subcutaneously or intramuscularly in the mid ventral part of the neck. Blood samples were collected before injection of the drug and every 4 days for 28 days after treated. The fluctuation in the blood constituents reported in his study was within the normal values reported by other workers. The animals did not show any side or adverse effects towards the drug investigated.

Chapter two

Materials and Methods

2.1 Survey of gastro intestinal helminths in equines

A field survey was conducted to study the prevalence of helminth parasites in donkeys (*Equus asinus*) and horses (*Equus Cabalus*). The aim of the survey was to report on the prevalence and intensity of infection with gastrointestinal helminth parasites in North Darfur State during the period October 2011 to May 2012.

2.2.1 The Study area

The study was conducted in North Darfur State Elfashir city, which is situated in the peripheral north western part of Sudan between 24 –27 longitudes East and 12 - 20 latitudes North (Figure 2.1).

2.1.2 Samples collection and examination

A total number of 1400 (900 donkeys, 500 horses) faecal samples were examined for parasites. Fresh faecal samples, were collected directly from the rectum of individual donkeys and horses in plastic bags (Figure 2.2), after labeling, the samples were transferred without delayed to El fashir diagnostic laboratory, Ministry of Animals Resources and Fisheries. Egg count was performed using modified McMaster technique (Anonymous, 1986) and the eggs were identified according to Soulsby (1982).

2.1.3 Intensity of infection

The severity of infection was obtained from the number of eggs per gram of faeces and was classified according to Soulsby (1982) as follows:

100-500 eggs per gram of faeces (mild infection)

500 to1000 eggs per gram of faeces (moderate infection)

>1000 eggs per gram of faeces (severe infection).



Figure (2.1) location of El fashir North Darfur State, Sudan

Figure 2.2: Faecal samples collection directly from rectum of a horse in El fashir veterinary hospital.

2.1.4 Parasitological Techniques

2.1.4.1 The modified McMaster Technique

- Three grams of faeces were mixed with 42 ml of tap water and the faecal suspension was then passed through 80 µm/square inch sieve to remove debris.
- The filtrate was collected in a clean dry bowl. 15 ml of this filtrate were transferred in to a centrifuge tube, centrifuged (Tiettical Zentrifugen, made china) for 2 minutes at 1500 rpm and the supernatant was then discarded.
- The sediment was suspended by gentle agitation and saturated NaCl was added until the volume became equal to the initial aliquot of the filtrate.
- The centrifuge tube was inverted several times to obtain an even suspension of the contents.
- The two chambers of the McMaster slide were filled using a clean Pasteur pipette.
- The average number of eggs present in these chambers was multiplied by 100 to obtain the number of eggs per gram of faeces (EPG).

2.2. Therapeutic efficacy, persistent effect, and treatment intervals of Abamectin against donkeys worm infestation

The objective of this study was to investigate the therapeutic efficacy, persistent effect and treatment intervals of Abamectin paste formulation at different dose regimens as an anthelmintic in donkeys harbouring natural worm infestation and to compare results obtained with that of ivermectin.

2.2.1 Experimental animals

In this study 24 donkeys, were selected from naturally infected animals. Infestation with gastrointestinal helminths was confirmed by faecal examination. Donkeys were kept in a house in Abushok area in El fashir city

North Darfur State (Figure 2.3). They were provided with clean water and allowed to feed together in one place.

2.2.2 Experimental drugs

The following drug formulations and trade marks were used for treatment of the infected donkeys:

1- Abamectin oral paste (Abamectin+Praziquantel) Wormnil paste (Mumbai India).

2- Ivermectin: Intermectin 1% injection

2.2.3 Experimental design

The animals were divided into four groups each of six. The first three groups were treated and the last group remained untreated as a control group. The animals in the three treatment groups received treatment as follows:

1- Abamectin treated group 1 (Aba1) received a single oral dose of abamectin as recommended by the dose (0.2 mg/kg body weight).

2- Abamectin treated group 2 (Aba2) received single oral dose of abamectin each day for five successive days as recommended by the dose of 0.2 mg/kg body weight.

3- Ivermectin treated group (Ivm) received a single dose by the route recommended by 0.2 mg/kg body weight.

Figure 2.3: Experimental animals housed in Abushok camp

2.2.4 Sampling schedule

Experiment extended to 77 days. Faecal samples were collected at 0 (before treatment), 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 days post treatment.

2.2.5 Data Analysis

2.2.5.1 Egg reappearance period (ERP)

Interval between treatments is usually based on the interval from treatment to reappearance of eggs in faeces. For the purpose of this study, the ERP has been defined as the interval between treatment and the time when the mean egg count in treatment group reached counts 200 or more eggs (arithmetic mean).

2.2.5.2 Calculation of efficacy and ERP

Percentage of efficacy of the treatments was calculated from the faecal egg count reduction, using arithmetic mean egg count with the following formula:

$$\text{FEER}\% = \frac{\text{Pre-treatment EPG} - \text{Post-treatment EPG}}{\text{Pre-treatment EPG}} \times 100$$

Modified McMaster technique (Anonymous, 1986) was used to count the eggs per gram (epg) of faeces. In order to estimate the time between treatment and the first reappearance of eggs in the faeces, the number of days was individually calculated for each donkey within a group as soon as the threshold was reached (equal or superior at 200 epg).

Figure 2.4: Collection of blood samples from jugular vein

2.3 Some biochemical parameters in sera from donkeys fmedication with Abamectin and Ivermectin

2.3.1 Collection of samples

Blood samples were collected from the jugular (Figure 2.4) using syringes and immediately transferred to containers (and they were allowed to clot, the clotted blood samples were centrifuged and sera were separated and stored at -20 °C until analyzed.

2.3.2 Biochemical methods

2.3.2.1 Total Serum Protein (TSP)

Total protein was measured using commercial kits (Bio system S.A, Barcelona, Spain). In alkaline medium the copper reacts with the peptide bonds of protein to form the characteristic pink to purple biuret complex. Sodium potassium tartarate prevent copper hydroxide precipitation and

potassium iodide prevents the auto-reduction of copper. The colour intensity is directly proportional to the protein concentration Gornall *et al*; (1949).

The principle of the test

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry. The change in colour was measured at 545nm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

2.3.2.2 Serum Albumin

Serum albumin is the most abundant protein in human plasma. It has three main function: it contributes towards maintaining the colloid oncotic pressure of plasma. It acts as non specific transport vehicle for many non polar compounds and it's a source of endogenous amino acids. Hyperalbuminemia results from several factors :reduced synthesis cause the disease to reduce absorption of amino acid due to mal absorption, increased catabolism as a result of inflammation or tissue damage; altered distribution between intravascular and extravascular space due to increased capillary permeability, overhydration or ascities , abnormal losses caused by renal disease (nephritis, diabetes mellitus, chronic glomerulonephritis, systemic lupus erythematosus (Doumas *et al* .,(1971).

The principle of the test

Albumin in the sample react with bromocresol green in acid medium forming a coloured complex that can be measured at 630nm using Jenway spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer.

2.3.2.3 Serum Total Bilirubin

Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucouronic acid to form a soluble compound. This conjugated bilirubin passes down the

bile duct and is excreted into the gastrointestinal tract. An un-conjugated, bilirubin bound form is also present in the circulation. It is insoluble and does not normally pass through the kidneys into the urine. An increase in bilirubin concentration in the serum or tissues is called jaundice. Jaundice occurs in toxic or infectious diseases of the liver e.g. hepatitis B or obstruction of the bile duct and in Rhesus incompatible babies. Useful information may be obtained by determining which form of bilirubin is elevated.

High levels of conjugated or direct bilirubin indicate that bile is not being properly excreted; therefore an obstruction may be present in the bile duct or gall bladder. Un-conjugated or indirect bilirubin can also be determined by subtracting the direct bilirubin level from the total bilirubin result. High levels of un-conjugated bilirubin indicate that too much haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin received (Tietz *et al.*, 1995).

The principle of the test:

Bilirubin was converted to coloured azobilirubin by diazotic sulfanilic acid and measure photometrically. Of the two fractions present in serum , bilirubin glucuronide and free bilirubin loosely bound to albumin. Only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect) in the results correspond to total bilirubin. The colour can be measured at 555nm using Jenway spectrophotometer using Egyptian commercial kits (Jenway 6305. V. /vis. Spectrophotometer U.k).

2.3.2.4 Serum Urea

Urea is synthesized in the liver as a product of the deamination of amino acids. Its elimination in the urine represents the major route for

nitrogen excretion. Elevated urea concentration in plasma is found as a result of a high protein diet, increased protein catabolism, after a gastrointestinal Haemorrhage, mild dehydration, shock and heart failure or treatment with glucocorticoids (pre-renal uremia). Post renal uremia is caused by condition that obstructs urine outflow: nephrolithiasis, tumor or prostatic hypertrophy. The usefulness of urea as an indicator of renal function is limited by the variability of its plasma concentration as a result of nonrenal factors.

The principle of the test

Urea in sample originates by means of the couple reaction described below complex that can be measured by spectrophotometry .



Serum urea concentration is measured by an enzymatic colorimetric method using a commercial kit (biosystem S.A. Barcelona. Spaina) according to Chaney and Marbach (1962).

The intensity of the developing colour was measured at 600 nm using Jenway spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.3.2.5 Serum creatinine

Creatinine is a catabolic end product of creatine (phosphocreatine), The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomerul (Small amounts are reabsorbed and are also secreted by the renal tubules). Creatinine measurement is used almost exclusively in the assessment of kidney function (impaired renal perfusion, loss of functioning nephorns) and in the monitoring of renal dialysis.

Principle of the method

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a

short period to a void interference. The colour was measured at wavelength 500 after 30 seconds (A1) and after 90 seconds (A2), using Jenway spectrophotometer (Jenway 6305 U.V. /vis. Spectrophotometer, U.K) according to Bartels and Bohmer(1971). (commercial kits were used (Biosystem S.A Costa Brava 30 , Barcelona (Spain).

2.3.2.6 Serum calcium

Serum calcium levels are influenced by changes in protein concentration. Increased calcium levels can be present in numerous disease states from dehydration to malignancies, eg. of lung or kidney. Low levels can also be clinically significant (Barnett *et al.*, (1973).

Test principle:

Calcium ions form a red –violet complex with the o- Cresolphthalein The intensity of the developing colour was measured at Hg 578 nm using Jenway spectrophotometer (Jenway 6305 U. V./vis. Spectrophotometer, U. K.).

2.3.2.7 Aspartate aminotransferase

The aminotransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. AST is found in higher concentration in the liver and hard muscle but it is abundant in skeletal muscle; kidney and pancreas. The serum concentration of AST is elevated in hepatitis and other form of hepatic diseases associated with necrosis infectious mononucleosis, cholestasis, cirrhosis metastatic carcinoma of the liver. AST concentration elevated after myocardial infarction, in skeletal muscle disease as progressive muscular dystrophy, in acute pancreatitis or hemolytic diseases (Friedman; 2002) . The formed colour was measured at 340nm using Jenway spectrophotometer (Jenway 6305 U. V. /vis.

Spectrophotometer, U. K). The commercial kits used were Biosystem S.A (Costa Brava 30 , Barcelona Spain).

2.4 The Statistical Analysis

Data obtained were analyzed using statistical package of social Sciences (SPSS). T test was used to determine the difference pre and post treatment values. Significant difference level was set as ($p < 0.05$).

Chapter three

Results

3.1 Survey of gastro-intestinal nematodes in donkeys and horses

Among the 1400 faecal samples the overall prevalence of infection with gastrointestinal nematodes was 24.6%. Only 25 faecal samples among the 500 faecal samples collected from horses were positive 5%, and 35.5% of the 900 faecal samples collected from donkeys were positive for helminth parasite (Table, 3.1).

As shown in table (3.2), 51.25% of the infected donkeys were harbouring single infection while the remaining (48.8%) harbored mixed infection. In horses, 92% were harbored single infection while 8% harbored mixed infection.

The current study showed with helminths that mild infection was encountered in 84% of horses and 69.7% in donkeys respectively, while moderate infection was encountered in 8% and 15.6% of horses and donkeys

, respectively, and severe infection was encountered in 8% and 14.7% in horses and donkeys, respectively.

Table 3.1: Overall prevalence of gastrointestinal nematodes in donkeys and horses in North Darfur State.

Donkeys				Horses				Total			
Positive		Negative		Positive		Negative		Positive		Negative	
No	%	No	%	No	%	No	%	No	%	No	%
320	35.5	580	64.4	25	5	47	95	345	24.6	1055	75.4
						5					

Table 3.2: Type of infection with gastrointestinal helminthes in donkeys and horses

Donkeys				Horses			
Single infection		mixed infection		Single infection		Mixed infection	
No	%	No	%	No	%	No	%
164	51.25	156	48.8	23	92	2	8

Table 3.3: Severity of infection with gastro-intestinal helminths in donkeys and horses

Horses						Donkeys					
Severe infection		Moderate infection		Mild infection		Severe infection		Moderate infection		Mild infection	
No	%	No	%	No	%	No	%	No	%	No	%
2	8	2	8	21	84	47	14.7	50	15.6	223	69.7

3.2 Therapeutic efficacy of Abamectin and ivermectin combination against gastrointestinal nematodes in donkeys (*Equus asinus*).

A total number of 24 donkeys naturally infected with gastrointestinal nematodes were used to evaluate the therapeutic efficacy, persistent effect and treatment interval of abamectin (single dose) , abamectin multiple dose paste formulation and ivermectin 1% injectable in doses rates of 200 µg per kg of live body weight.

Animals were divided in four groups of 6 donkeys each. Faecal egg count was performed 3 days before treatment. Means and standard deviation was calculated in abamctin single dose (group one), abamctin single dose for five successive days (group two), control (group three) and single dose of injectable ivermectin solution were found, 1766.7 ± 294.4 , 1116.7 ± 172.24 516.7 ± 75.3 and 550 ± 54.77 respectively.

Faecal egg count performed one week after treatment and then every week for 10 consecutive week. Seven days post treatment all abamectin (single dose), abamectin for five consecutive days and ivermectin single dose were found negative to egg of parasites, parasite eggs reappeared on day 49, in abamectin paste formulation (singl dose) and on day 56 for both abamectin paste formulation for five consecutive days and ivermectin injectable solution. The percentage of efficacy of three medicated groups abamectin single dose, abamectin 5 doses and ivermectin against nematodes parasites were found to be 100% on day 14 post treatment. The persistent effect of single dose of abamectin was continuous to day 70, while in both abamectin 5 doses and ivermectin single dose extended to day 77. The treatment interval was 66.5 ± 52 in abamectin single dose and 72.3 ± 32.7 in both abamectin 5 doses and ivermectin single dose. No adverse reactions were observed during the experimental period (Table 3.5).

Tables 3.5: Percentage efficacy and persistent of single dose of abamectin, abamectin for five successive days and avermectin at 14 days to 77 days post treatment

Days	Aba1 M±SD	Aba2 M±SD	Ivm M±SD	Control M±SD
0	1766.67±294.39	1116.67±172.24	550±54.77	516.7±75.28
0	1683.33±292.69	916.67±371.03	483.33±40.82	533.33±81.65
0	1550±251.00	933.33±314.21	500±0.00	516.67±75.28
7	0±0	0±0	0±0	516.66±639.94
14	0±0	0±0	0±0	516.66± 40.82
21	0±0	0±0	0±0	550±54.77
28	0±0	0±0	0±0	550±83.67
35	0±0	0±0	0±0	600±63.67
42	0±0	0±0	0±0	633.33±103.28
49	0±0	0±0	0±0	816.66±116.90
56	33.33±435.51	0±0	0±0	883.33±213.70
63	183.33±435.51	50±54.77	50±109.54	1266.66±436.65
70	200±0.00	100±109.54	100±109.54	1333.33±458.98
77	200±0.00	200±0.00	200±0.00	1416.66±503.65

3.3 Some biochemical parameters in donkeys medicated with Abamectin and Ivermectin

Table (3.6 to 3.12), show the changes in total serum protein , albumin, urea, creatinine, bilirubin, calcium and AST, following administration of abamectin paste formulation at different doses and of ivermectin injectable solution.

Table (3.6), animals in treatment group (abamectin single dose paste formulation), show significant ($p < 0.05$) increase in the total serum protein on days, 7, 14 and 39 post treatment when compared to pretreatment level. In group 2 abamectin paste formulation for five successive days was significant ($p < 0.05$) increase in the total serum protein on day 14 and 39 post treatment when compared to pretreatment level, while in group 3 (ivermectin single dose injectable) , there is significant ($p < 0.05$) increase in total serum protein on day 14 post treatment when compared to pretreatment level.

Table (3.7), animals in group 1 (abamectin single dose paste formulation), showed significant ($p < 0.05$) increase serum albumin concentration on days 7,14 and 24 post treatment when compared to day zero, while animals in group 2 (abamectin single dose for five successive days paste formulation) had significant ($p < 0.05$) increase in serum albumin on day 14 post treatment when compared to day zero and donkeys in group 3 (ivermectin single injectable solution) had significant ($p < 0.05$) increase in serum albumin concentration on day 24 post treatment when compared to day zero.

Table (3.8), animals in treatment group 1 (abamectin single dose paste formulation), had significant ($p < 0.05$), increase in serum urea concentration on days 7, 10, 14 and 24 post treatment when compared today zero, in group 2 (abamectin single dose for five successive days paste formulation) was significant ($p < 0.05$) increase in serum urea concentration on day 10, 14, 24 and 39 in post treatment when compared to day zero, while in group 3 (ivermectin single doses injectable) was significant ($p < 0.05$) decrease in serum urea concentration on day 39 post treatment when compared to pretreatment level .

Table (3.9), show that animals in treatment group 1 (abamectin single dose paste formulation), had significant ($p < 0.05$) increase in serum creatinine concentration on days 7, 10 , 14 and 39 post treatment when compared to day zero, animals in group 2 (abamectin paste formulation single dose for five successive days) had significant ($p < 0.05$), increase in serum creatinine concentration on day 7, 10, and 24, post treatment when compared to day zero, while animals in group 3 (ivermectin injectable single solution doses) had significant ($p < 0.05$) decrease in serum creatinine concentration on day 24 post treatment when compared to day zero.

Table (3.10), showed that donkeys in group 1 treatment group 1, had significant ($p < 0.05$) increase in serum bilirubin concentration on days 10, 14, 24 and 39 post treatment when compared to day zero , animals in group 2 showed significant ($p < 0.05$), decrease in serum bilirubin concentration on day 7, post treatment when compared to day zero, while animals in group 3 animals had significant decrease on day 7 post treatment when compared to day zero, while animals had significant increases on day 14, 24, and 39 post treatment when compared to day zero.

Table (3.11), show that animals in group 1 had treatment group significant ($p < 0.05$) increase serum calcium concentration on days 7 in post treatment when compared today zero, animals in group 2 had significant decrease in serum calcium concentration on day 7 and 10 post treatment when compare to day zero, while animals showed significant ($p < 0.05$), increase on day 7, 10, 24 post treatment when compared to pretreatment level, while group 3 had significant increase in serum calcium concentration on day 7 and 10 post treatment when compared to pretreatment.

Table (3.12), showed that donkeys in group 1 had significant decrease in serum AST concentration on day 7, while donkeys showed significant ($p < 0.05$), increase in serum AST concentration on day 10 and 39 post treatment when compared to day zero level, while animals in group 2 show significant

decrease in serum AST concentration on 7, 10 and 39 post treatment when compared to pretreatment level, and significant increase in serum AST concentration on day 14, while animals in group 3 had significant ($p < 0.05$) increase in serum AST concentration on day 7, 10, 14, 24, and 39 post treatment when compared to pretreatment level.

Table 3.6: Effect of treatment with abamectin (single dose), abamectin (multiple dose regime) and ivermectin (single dose) on donkeys serum total protein concentration (g/l).

Day	Aba1	Aba2	Iver
	Mean±SD	Mean±SD	Mean±SD
Day 0	69.53±13.99	64.37±12.67	67.45±5.65
Day7	80.43±11.55*	73.18±11.31	71.28±10.22
Day10	72.28±17.98	70.30±17.25	72.47±15.54
Day14	87.58±13.81*	86.65±8.85 *	91.02±15.7*
Day 24	63.18±5.87	67.22±5.68	64.57±12.89
Day39	*80.80 ±7.25	79.27±8.89*	66.82±8.10

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment.

Table 3.7:Effect of treatment wabamectin (multiple dose regime) and ivermectin (single dith abamectin (single dose), ose) on donkeys serum Albumin concentrationg/I)

Days	Aba1	Aba2	Iver
	Mean±SD	Mean±SD	Mean±SD
Day o	23.06±3 .75	23.17±1 .53	24.47±2. 57
Day7	25.62±2.43*	24.38±4.64	24.97±4.41
Day10	23. 12±1 .13	23. 15±1 .98	23.43±3 .07
Day14	26.18±1 .99*	27.12±10.28*	23.25±2.87
Day24	26 .53±3 .42 *	24.65±1.34	26.70±2.58*
Day39	20 .48±5.96	24.15±2.2	24.15±2.27

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment.

Table 3.8: Effect of treatment with abamectin (single dose), abamectin (multiple dose regime) and ivermectin (single dose) on donkeys serum urea concentration (mmol/L)

Day	Aba1	Aba2	Iver
	Mean±SD	Mean±SD	Mean±SD
Day 0	3.82± 0. 90	3.95±0 .48	4.37±0.63
Day7	4.65±0.63*	3.88±0.61	4 .47±0 .41
Day10	4.67 ±0.59*	4.40±0 .16*	4.31 ±0.54
Day14	4.25±0 .57*	4.17± 0.37*	4.23±0.81
Day24	4.37±0 .43*	4.63±1 .17*	4. 08±0.61
Day39	3.05±1.11	4.07±0.60*	3.22±0. 89*

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment

Table 3.9:Effect of treatment with abamectin (single dose), abamectin (multiple dose regime) and ivermectin (single dose) on donkeys creatinin concentration (mmol/L)

Day	Aba1	Aba2	Iver
	Mean±SD	Mean±SD	Mean±SD
Day 0	72.809 .99	71. 29 ±59.7 8	85.35±7.69
Day7	82 .87±12.71 *	82.60 ±18.72 *	84.55±5.45
Day10	81.48 ± 19.00 *	82.30±9.09 *	84 .35±13 .67
Day14	83.22±11 .12 *	69.43± 9.06	81.68±16.25
Day24	78 .57± 14.50	84.15±12 .17 *	78.67 ±12.05 *
Day39	80.70±15.6 *	78.68±15.74	85.15±7.56

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment

Table 3.10: Effect of treatment with abamectin (single dose), abamectin multiple dose regime and ivremectin single dose on donkeys serum bilirubin concentration (mmol/L)

Day	Aba1	Aba2	Iver
	Mean±SD	Mean±SD	Mean±SD
Day 0	0.42±0.36	1.02±0.57	1.68±0.43
Day7	0.54±0.44	0.32±0.28 *	0.26±0.17*
Day10	1.67±0.33*	1.72±0.503	1.98± 0.99
Day14	1.12±0.64*	1.68± 0.93	2.02±0 .37*
Day24	1.12±0.64*	1.68±0.93	2.02 ±0.37*
Day39	1.81±0.43 *	1.75±0.51	2.88±1.46*

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment.

Table 3.11: Effect of treatment with abamectin (single dose), abamectin multiple dose regime and ivremectin single dose on donkeys serum calcium concentration (mmol/L)

Day	Aba1	Aba5	Iver
	Mean±SD	Mean±SD	Mean±SD
Day 0	1.50±0.50	1.70±0.78	1.34±0.42
Day7	2.18±0.58 *	2.03±0.39*	2.07±0.36*
Day10	1.90±0.49	2.12±0.51*	2.07±0.50*
Day14	1.97±0.51	1.98±0.46	1.73±0.43
Day24	1.87±0.47	2.07±0.29*	1.40±0.49
Day39	1.64±0.72	1.56±0.30	1.38±0.21

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment.

Table 3.12: Effect of treatment with abamectin (single dose), abamectin multiple dose regime and ivremectin single dose on donkeys serum AST concentration (IU/L)

Day	Aba1	Aba2	Iver
-----	------	------	------

	Mean±SD	Mean±SD	Mean±SD
Day 0	133.67±40.79	155.83±24.3	122.00±37.73
Day7	100.33±21.62 *	102.17±36.79 *	147.83±35.55 *
Day10	148.0±50.69 *	123.33±13.02 *	114.50±57.28 *
Day14	135.00±36 .09	174.83±3.76*	133.00±28.82 *
Day24	138.50±37.56	154.67 ±27.34	170.33 ±36. 66*
Day39	149.33±21.65*	130.33±18.67 *	148.33±14. 61*

- The means on same column that have an asterisk are significantly (P<0.05) different from pretreatment.

Chapter four

Discussion

Large number of intestinal parasites have been reported in donkeys in previous investigation in six African countries including Ethiopia, Kenya,

Zimbabwe, Burkinafaso, Chad and Morocco. Intestinal parasites were considered as problem in donkeys causing poor body condition, reduced power output, poor reproductive performance and short lifespan (Pandey *et al.* ;1994).

In this study the overall prevalence of nematode infection for both donkeys and horses were found to be (24.64%), this result is in close agreement with that obtained by (Sawsan *et al.*, 2008) in South Darfur State (29.9%), similar results were also obtained by Kheir and Kheir (1981) in Bahr El Arab 22% , in Sennar –Sudan. (El Dirdiri *et al*; (1986) reported comparable percentages of infection with gastro intestinal worms (27%). However, the percentage recorded in the current study were by far less than that reported in Nyala town 58%. Kheir and Kheir (1981) who examined only390 animals. This may attributed to the large number of animals examined in study (1400 animales).

The prevalence of gastrointestinal nematodes in horses recorded in this study was 5%, which was less than that reported by (Sawsan *et al.*,2008) 15.73%, and Kheir and Kheir (1981) in Bahr El Arab 18.5%. This might be attributed to management differences and seasons. Horses investigated in this study received good health care from owners, and high economic value of horses when compared to donkeys.

The Result obtained for prevalence of helminths in donkeys (35.5 %) was similar to result recorded by (Sawsan *et al* ;2008), in Nyala town- Sudan 37.85%, but lower to prevalence reported by Seri *et al.*, (2004) in Khartoum State (70.1%). Ibrahim *et al.*, (2011) reported in khartoum 63.64%, and Ahmed (2008) in Nyala town performed necropsy to donkeys and reported (97.78%) prevalence, while Kheir and Kheir (1981) reported

that the overall prevalence of infection with nematode parasites was found higher in urban animals (58%) than in animals kept in nomadic areas (22%).

Infection with single nematode species in donkeys was higher than multiple infection. This finding agree with the findings of Seri *et al.* (2004), in Khartoum State and kheir and kheir (1981) in South Darfur State mild infection was reported in 69.7% of the donkeys, while both moderate and severe infection was reported in 15.6% and 14.7%, respectively. The finding were comparable to those recorded by Sawsan *et al.*, (2008) being 81.25%, 7.89% and 10.86%), for mild, moderate and severe infection respectively. Ibrahim *et al.*, (2011) reported similar results for mild, moderate and severe being 26.89%, 12.61 and 53.78 respectively in Khartoum state.

The results obtained were also higher than those reported by Seri *et al.* (2004) in donkeys 58.6%, 21.9%, and 19.5% for mild, moderate and severe infection, respectively. Ayele *et al.*, (2006) reported different findings being 6.2% 3.8% and 81.7% for mild, moderate, and severe infection, respectively in Ethiopia. This might be due to lack of veterinary services, management system and the small number of animals examined (339). In horses the values obtained by Sawsan *et al.*, (2008), were 82.35%, 8.82% and 8.82 for mild, moderate and severe infection, respectively.

Therapeutic efficacy of abamectin paste (single dose), abamectin (for five successive days), was 100% recorded for both groups on day 7 and 14 post treatment. The drugs persistent effect in abamectin (single dose)

extended to day 49 post treatment, while in both abamectin (multiple doses) and ivermectin (single dose) was extended to day 56 post treatment.

The parasites eggs were appeared and the eggs count reached 200 eggs per gram on day 70 in abamectin single dose and to day 77 in both abamectin multiple dose and ivermectin single dose post treatment.

These findings agree to Williams *et al.*, (1992) who evaluated the efficacy of abamectin against natural infections with gastrointestinal nematodes and lung worm of cattle with special emphasis on inhibition of early fourth stage larvae of *Ostertagia ostertagi*, , he found that the efficacy of abamectin at 6 and 14 days post treatment was greater than 99% efficacy against nematodes parasite and lungworm larvae. The results obtained disagree with Alka *et al.*, (2004) who used abamectin against ivermectin resistant strain of *Trichostrongyles colubriformis* in sheep at 0.2mg/kg body weight. He found faecal egg reduction in sheep 10 days post-treatment also Entrocasso *et al.*, (1996) who compared the efficacy of the same four macrocyclic lactones in maintaining reduced faecal egg count in cattle grazing naturally in pasture in Argentina, Brazil and Colombia. They observed observed reduction in mean cumulative faecal egg count in treated animals 63 days post treatment and Meeus *et al.*, (1997) in Zambia in cattle using abamectin at dose rate 0.2 mg/kg against *Cooperia* and *Heamonchus* eggs and found that parasites eggs were reduced in faecal egg count 48 days post treatment with cut point 100 epg.

The efficacy of ivermectin was 100% on day 7, the faecal eggs count reduction, agree to Seri *et al.*, (2005), Sawsan *et al.*, (2010), Fangama *et al.*, (2013) reported that 100% faecal eggs count reduction using

ivermectin at a dose rate 0.2mg/kg subcutaneously and agree to finding in horses reported by Costa *et al.*, (1998).

The total protein values in three treatment groups showed significant ($p<0.05$) difference post treatment in values when compared to day zero. Total protein concentration in three medicated groups ranged between 71.3 ± 10.2 to 91.2 ± 15.7 g/L, values and in agreement who reported by Seri *et al.*, (2006), who also reported (73.3 ± 2.1) g/L post medicated groups compared to zero.

The albumin concentration showed significant ($p<0.05$) difference post treatment values compared to day zero values. Albumin concentration in the three medicated groups ranged between 20.48 ± 5.96 to 27.12 ± 10.28 g/L and were in close agreement with (Seri *et al.*; 2006), who reported values ranging from (27.9 ± 2.8) to (43.5 ± 1.9) g/l post medicated animals.

The urea concentration showed significant ($p<0.05$) differences post treatment groups when compared to pretreatment groups. Urea concentration in three medicated groups ranged from 3.82 ± 0.90 to (4.47 ± 0.41) mmol/L and were also close to values reported by by (Seri *et al.*; 2006), who reported values ranging from 1.71 ± 0.27 to 5.52 ± 0.43 mmol/L.

Calcium concentration showed significant ($p<0.05$) differences in post treatment groups compared to pretreatment groups. Calcium concentration in three medicated groups ranged 1.90 ± 0.49 to 2.2 ± 0.58 mmol/L. These results were in agreement with the values recorded by Seri *et al.* ;2006, who also reported 2.23 ± 0.08 to 2.60 ± 0.04 mmol/L in post medicated animals.

Creatinine concentration showed significant difference post treatment groups compared to pretreatment groups. Creatinine concentration

in three medicated groups ranged from 69.43 ± 9.06 to 85.2 ± 7.7 mmol/L and were in agreement with normal values reported by De Aluja *et al.* (2006).

Bilirubin concentration showed significant difference in post treatment groups compared to pretreatment groups. Bilirubin in three medicated groups ranged from 0.26 ± 0.17 to 2.89 ± 1.46 mmol/L, and were in agreement with normal values reported by De Aluja *et al.* (2006).

AST concentration showed significant differences post treatment groups compared to pretreatment groups. AST in three medicated groups ranged from (100.33 ± 21.62) to (174.83 ± 3.76) IU/L, and were within the reference ranges of previous reports (French and Patric 1995) but were slightly lower than the normal values reported by De Aluja *et al.* (2006). All the findings proved the abamectin and ivermectin medication did not produce adverse effect in donkeys.

Chapter five

Conclusion and Recommendations

5.1 Conclusion

The present study provided a comprehensive description of the prevalence of equine internal parasites, abamectin and ivermectin, efficacy ,persistant effect, treatment interval and biochemical analysis of blood serum in North Darfur State as below

Survey was achieved in October 2011 to May 2012 in donkeys and horses 900 and 500 respectively. The overall prevalence of nematodes infection in donkeys and horses was 24.64%, while the prevalence in donkeys and horses 35.5%, 5% respectively. It is to be concluded that the prevalence of internal parasites was low compared to previous studies.

The therapeutic efficacy of abamectin (single doses), abamectin 5 doses and ivermectin was recorded 100%. Abamectin and ivermectin had no deleterious effects on the treated donkeys and the persistent effect of abamectin extended to day 70, while multiple dose with abamectin paste formulation and ivermectin single dose extended both today 77.

5.2 Recommendations

1- Donkeys should be include in the priority lists of research to develop sustainable integrated diseases prevention program for control of diseases..

2- Adopt a nationwide program for elimination and control of infection with internal parasites to prevent reinfection of pasture.

3- Use of anthelmintic paste should be adopted to evaluate its efficacy for treatment and control of parasitic infection in equidae.

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