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Prevalence, Risk Factors and Prophylactic Measurements of Helminth Infestation in Draught Donkeys in West Kordofan State

الإنتشار وعوامل الخطر والقياسات الإتقائية للإصابات بالديدان في حمير الجر في ولاية غرب كردفان

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بسم الله الرحمن الرحيم

والأنعامَ خَلَقَها لَكُم فِيهَا دِفءٌ ومَنَافعُ ومِنهَا تَأكُلُونَ * ولَكُم فِيهَا جَمَالٌ حِينَ تُرِيحُون وحِينَ تَسرَحُونَ * وتَحمِلُ أثقَالَكُم إلَى بَلَدٍ لّم تَكُونُوا بَالِغِيهِ إلّا بِشِقِّ الأَنفُسِ إنّ رَبّكُم لَرَءُوفٌ رَّحِيمٌ * والخَيلَ والبِغَالَ والحَمِيرَ لِتَركَبُوهَا وزِينَةً ويَخلُقُ مَا لَا تَعلَمُونَ *

صدق الله العظيم

سورة النحل الآيات (5 ، 6 ، 7 ، 8)

DEDICATION

To

All members of my family

My supervisors

My friends and colleagues

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Abstract

The study was conducted to assess the prevalence rates of helminthic infestations, its associated risk factors and to investigate prophylactic measurements for the control of helminth infestation in draught donkeys in Ghebaish and Alfula localities and selected surrounding villages of West Kordofan State. The study included examination of (1200) fecal samples, (600) blood samples for PCV. Besides (600) questionnaires were filled by donkey owners.

Faecal samples examination revealed (9) different pathogenic helminth throughout the year, giving an overall prevalence rate 38.25% (20.25% for Ghebaish and 18% for Alfula). The majority of the infected animals were found with *strongylus* representing (59.91%) followed by mixed infection, *Dictyocaulus, Strongyloids, Cyathostomes, Oxyuris, Parascaris, Anoplocephla, Paranoplocephla* and *Trichostrongylus* constituting (16.78%), (6.54%), (5.01%), (3.49%), (3.49%), (2.61%), (1.09%), (0.65%) and (0.44%), respectively. The helminth infection rate in Ghebaish locality was found higher representing (53%), whereas, in Alfula locality was (47%). The overall mean egg per gram count (epgc) was 704.14±116.59 with a range of 50 - 10200 (epg). The infection was found (68.85%) for mild, (17.86%) for severe and (13.29%) for moderate infections.

Most of infections were found during dry hot season (poor vegetation season) representing (51.63%) followed by wet and dry cool constituting (24.84%) and (23.53%), respectively. *Strongylus spp* was found predominant in the three seasons, high in the dry hot followed by dry cool and then wet season. The prevalence of *Strongylus spp* and mixed infection were found to be significantly higher in the two localities mainly in Ghebaish during the dry hot season. The value of mean packed cell volume in study area was found to be 32.38±7.37. The minimum and maximum values of PCV were 17 and 50 respectively. The mean PCV difference was not statistically significant.

Questionnaires analysis for risk factors revealed; the prevalence rate of helminth infection of donkeys of owners who responded to the questionnaire was (38.50%). The majority of the donkeys were found in good and moderate body condition. The Highest helminth infections were found among age group 6 – 10 years representing (68.83%), followed by >10 years (19.91%) and ≤ 5 years (11.26%). Whilst, there was no statistically significant difference in infection according to sex. Helminth infestations were found high in donkeys with bad body condition, fed indoor, tethered with sandy bedding. The great majority of the infected draught donkeys were found related to the owners who were not aware of the presence of helminth infections and the use of anthelmintic treatments, and had no clear idea about the sources and response to the treatment.

The therapeutic efficacy percentage of the four treatment groups used was 100% 14 days post-treatment. The persistent effect of Moxidectin was continuous up to D 91, while in Ivermectin, Doramectin and Albendazole extended to D 84. The treatment interval was 98.7±6.96 in Moxidectin, 88.90±8.76 in Ivermectin, 88.20±5.90 in Doramectin and 83.22±14.19 in Albendazole.

Accordingly, due to limitless roles and importance of donkeys in livelihood and social life of human, it is recommended that government and non-governmental organizations should coordinate and collaborate in joint multidisciplinary plans to improve the health and welfare of donkeys in Sudan. Further researches on epidemiology of donkey diseases are recommended.

المستخلص

أُجريت هذه الدراسة لتقييم معدلات إنتشار الإصابات بالديدان الطفيلية، تقييم عوامل الخطر المرتبطة بها والتحقق من القياسات الإتقائية بغرض التحكم في هذه الإصابات في حمير الجر في محليتي غبيش والفولة والقرى المجاورة لهما بولاية غرب كردفان. شملت الدراسة (1200) عينة رَوَث و (600) عينة دم لحجم الخلايا المُكدّسة (PCV). وأيضاً (600) إستبيان تم ملئها بواسطة مالكي الحمير.

أظهر فحص عينات البراز (9) ديدان مختلفة مسببة للأمراض خلال العام، أعطت معدل إنتشار عام 38.25% (20.25% لغبيش و 18% للفولة). الأغلبية من الحيوانات المصابة وُجدت بديدان (*Strongylus spp*) تمثل (59.91%) تليها الإصابة المختلطة، *Dictyocaulus*، *Strongyloids*، *Cyathostomes*، *Oxyuris*، *Parascaris*، *Anoplocephla*، *Paranoplocephla* و *Trichostrongylus* بنسب (16.78%)، (6.54%)، (5.01%)، (3.49%)، (2.61%)، (1.09%)، (0.65%) و(0.44%)، على التوالي. من بين الحيوانات المصابة، وُجد أن أكثر الإصابات في محلية غبيش بنسبة (53%)، بينما بلغت في محلية الفولة (47%). بلغ متوسط عد البيض بالجرام / روث 704.14 ± 116.59 بنطاق 50 ـــ 10200 بيض/جرام. وُجِدت الإصابات الخفيفة كأعلى نسبة حيث بلغت 68.85% تليها الشديدة بنسبة 17.86% ثم الإصابات المعتدلة بنسبة 13.29%.

وُجِدت أغلب الإصابات في موسم الصيف (يفتقرللغطاء النباتي) بنسبة (51.63%) يليه موسم الخريف (wet hot season) ثم موسم الشتاء (dry cool) التي تشكل (24.84%) و (23.53%)، على التوالي. أيضاً أظهرت النتائج أن ديدان *Strongylus* هي الغالبة في كل المواسم، عالية في موسم الصيف يليه الشتاء ثم الخريف. معدل إنتشار ديدان *Strongylus* والإصابة المختلطة جاءت بائنة إلى حد كبير في المحليتين وخاصة في غبيش خلال فترة الصيف. أوضحت نتائج فحص الدم أن متوسط قيمة حجم الخلايا المُكدّسة في منطقة الدراسة 32.38 ± 7.37. ووُجِد أدنى قيمة لحجم الخلايا المُكدّسة 17 وأعلاها كانت 50. وإتضح أن متوسط حجم الخلايا المُكدّسة لا تختلف إحصائياً إختلافات معنوية كبيرة.

كشفت نتائج فحص الروث أن الحمير التي يمتلكها الأشخاص الذين إستجابوا للإستبيان مصابة بمعدل (38.50%). أوضحت نتائج الإستبيان في شأن عوامل الخطر أن أغلب الحمير في حالة صحية معتدلة وأخرى في حالة جيدة. أعلى نسبة إصابة بالديدان كُشِفت في الحمير ذات الفئة العمرية 6 – 10 سنوات بنسبة (68.83%) تليها أكبر من 10 سنوات ثم years 5 ≤ بنسب (19.91%) و (11.26%) على التوالي. بينما لا يوجد فرق (مغزى) إحصائي ذو دلالة على الإصابة بناءاً على

الجنس. وُجِدت أعلى إصابات للديدان الطفيلية في الحمير ذات الحالة الصحية السيئة، التى تعلف داخلياً ومربوطة على أرض رملية. علاوة على ذلك الغالبية العظمى من الحمير المصابة وُجِد أن مالكيها لا يدرون بوجود إصابات ديدان أو إستخدام طاردات الديدان، وليس لديهم فكرة واضحة عن مصدر العلاج والإستجابة له.

بلغت نسبة فعالية العلاج للأربع مجموعات 100% في 14 يوماً بعد إعطاء العلاج. الأثر المستمر للموكسيدكتين إستمر حتى اليوم ال91، بينما إستمر في الأيفرمكتين والدورامكتين والألبندازول حتى اليوم ال 84. كما أظهرت نتائج التجربة العلاجية أن: الفاصل الزمني للعلاج في الموكسيديكتين كان 98.70± 6.96، في الأيفرمتكتين 88.90 ± 8.76، في الدورامكتين 88.20 ±5.90 وفي الألبندازول 83.22 ± 14.19.

بناءاً على ماسبق ونسبة للأدوار غير المحدودة وأهمية الحمير في سُبُل كسب العيش والحياة الإجتماعية للمجتمعات، يُوصى بأهمية التنسيق والتعاون بين الحكومة والمنظمات غير الحكومية من أجل عمل خطط مشتركة متعددة الأغراض بغرض تحسين صحة ورعاية الحمير في السودان. كما يُوصى بإجراء بحوث متقدمة في وبائية أمراض الحمير.

Table of Contents

List of Tables

List of Figures

List of plates

Introduction

The diverse a groclimatic conditions, animal husbandry practices and pasture management have been shown to largely determine the incidence and severity of various parasitic diseases in different geographical regions around the globe (Kaplan, 2004).

Livestock is the largest subsector of the Sudanese domestic economy and is a growing contributor to exports. To a remarkable extent, the Sudanese economy is based on a combination of mobile and sedentary pastoral and agro-pastoral production by farming and herding households in almost every region and state (Behnke, 2012). Although donkeys are both widespread and economically important to their owners, they are rarely studied and are not usually the object of improvement, development or loan schemes. Donkeys are not conventional sources of meat or milk, and their uses as pack animals and for traction do not fit within the stereotyped perspectives of livestock agencies. Nonetheless, they remain essential to the subsistence strategies of many communities in semi-arid regions, relieving families of repetitive and energy consuming tasks. Moreover, they stay healthy on a varied and often poor-quality diet and require little management (Blench, 2012).

Donkeys in Sudan have been used for different purposes, among which income generation is the main source of living particularly among rural population. Gastrointestinal parasites do adversely affect the income generation by infecting these working donkeys. In Sudan the significance of helminth infestation in donkeys has been reported in several studies (Seri *et al.*, 2004; Najat, 2006; Sawsan *et al*., 2008; Adam *et al*., 2013; Abdurhman *et al*., 2016). These studies reported the importance of donkey in livelihood and different prevalence rates of gastrointestinal parasites, particularly helminth among donkeys from some parts of the Sudan. Nevertheless, and to the best of our knowledge no study has been done in West Kordofan regarding the association between donkeys and helminth infection.

In Sudan donkey population approximately 7, 5 million heads, 54.15% of it exists in the western States (Kordofan and Darfur States) (MARF, 2009). These significances dependence indicate the potential impact of donkeys on daily life of people of West Kordofan in terms of financial influence and health burden **(Appendices 5, 6, 7, and 8)**. Therefore, the aim of the present study was to assess the prevalence rates of helminthic infestation, its associated risk factors and to investigate prophylactic measurements for the control of helminth infestation in draught donkeys in West Kordofan State.

General objective

To investigate the prevalence as well assessment of risk factors associated with parasitic helminth in draught donkeys, and to study prophylactic measurements for the control of helminth infestation in donkeys in West Kordofan State.

Specific objectives

Due to the shortage of information about draught equines in west Kordofan this study was designed to:

1- Study the prevalence of helminth infestation in draught donkeys in West Kordofan State.
2- Report on the risk factors associated with helminth infestation in draught donkeys.
3- Investigate the therapeutic efficacy, persistent effect and treatment intervals of drugs under investigation.
4- Come out with recommendations that can contribute in designing and implementing future control practices of gastrointestinal parasites in draught donkeys in West Kordofan.

Chapter one

Review of Literature

1.1 Background

Veterinary parasitology is the study of animal parasites, especially relationships between parasites and animal hosts, and their interactions (Gregory, 2010; Tripathy, 2015). A parasite is an organism living in or on another living organism obtaining from it part of or all of its organic nutrient, and commonly exhibiting some degree of adaptive structural modification; such an organism that causes some degree of real damage to its host (Bush *et al.*, 2001).

Parasites are as a fascinating group of invertebrates that have evolved and developed with many of their hosts and may or may not produce clinical disease, depending on a variety of environmental, ecological, immunological, physiological, and managerial factors that influence the host parasite relationship (William, 2001). The parasite derives nutritional benefit from the host and is usually considered harmful to the host, although the degree of harm can vary greatly (Rickard, 2001).

Anywhere in the world, hosts, parasites and the local environment are intimately linked. Parasites are totally dependent on these linkages for transmission and for persistence in host populations. In many situations, there is balance between the needs of the hosts and of the parasites, but circumstances can shift to favor either the hosts, with a possibility of parasite loss or extinction, or the parasites, with the risk of host morbidity and or mortality. For many animal populations, these effects on hosts and parasite illustrate the potentially critical role for parasites in the structure and function of local ecosystems, are they savannah, swamp, forest, mountain, or pasture (Polley, 2010). The structure and type of the grass depend upon soil type, and that determine the degree of formation of the (mat) between the soil and the grass. The mat is mainly found at the old grazing lands, and it keeps the high humidity for weeks in dry seasons. Presence of this humidity and air pockets in the mat decreases the change of temperature, which helps in presence and growth of larvae of parasites. (Magzoub, 2002).

Climate change, altered land use, and the search for resources are among the impacts of human activity on ecosystems around the world. A possible result of these impacts is changed patterns of diseases in domestic and free-ranging animals, and in people. On occasion, and despite the resilience inherent in many ecosystems, these disturbances lead to the emergence of new parasitic diseases or to the re-emergence of diseases that were common in the past (Polley, 2010).

Helminth parasites (Nematodes, Cestodes and Trematodes) are ubiquitous among domestic animals and wildlife in ecosystems around the world. Their life cycles depend on close linkages with their hosts and the environment, and transmission for many species depends on behaviors and resilience. Helminth can reduce production (of meat, milk, and wool), can cause clinical disease and death, and many have more subtle effects. The significance of helminth depends primarily on parasite and host species, levels of infection, and the host's overall health status (Rickard, 2001).

The life cycles of parasitic helminth are either direct or indirect. With direct life cycles, characteristic of many nematodes important in veterinary medicine. The adult parasites live and develop only in a mammalian or avian definitive host, in which they undergo sexual reproduction, and the parasite is transmitted among these hosts by free living stages in the environment. Indirect life cycles, characteristic of other nematodes and of all Cestodes, Trematodes and Acanthocephalans, involve a definitive host, and often the environment, but also require one or more (sequential) intermediate hosts in which immature parasites undergo essential development and may reproduce asexually (Polley, 2010).

1.1.1. Helminth parasites

The name helminth is derived from the Greek words helmins or helminthos which means, a worm, and is usually applied only to parasitic and non parasitic species belonging to the phyla Platyhelminthes (flukes, tapeworms and other flatworms) and Nemathelminthes such as round worms. Helminth are mainly belong to three major groups, namely Nematoda, Cestoda and Trematoda (Soulsby, 1982). Furthermore, (Polly, 2010) added; helminth important in veterinary medicine are in four distinct taxonomic groups: Nematodes (commonly known as roundworms); Cestodes (commonly known as tapeworms); Trematodes (commonly known as flukes); and Acanthocephalans (commonly known as thorny-headed worms).

As important helminth parasites, Clark (2008) stated that; Strongyles are classified into two groups: large and small. The large strongyles that infect equines include three *Strongylus* spp; *Strongylus vulgaris*, *Strongylus edentatus*, and *Strongylus equinus*, and several *Triodontophorus spp*. Small strongyles consist of more than 50 different species from several different genera. However, most of the small strongyles that infect equines belong to four genera: Cyathostomum, Cylicocyclus, Cylicodontophorus, and Cylicostephanus. Although it is still acceptable to refer to these parasites as small strongyles, the World Association for the Advancement of Veterinary Parasitology has recommended that they be referred to as Cyathostomins or Cyathostomes.

Previous studies and observations conducted have pinpointed helminthic parasites as being a major health hazard, limiting the overall performance of equines. Among the helminthes, strongyles (large and small strongyles), *Trichostrongylus axei*, *Triodontophorus species*, *Trichonema species*, *Parascaris equorum*, *Anoplocephala species*, *Dictyocaulus arnfieldi*, and *Fasciola species* are the most known devastating parasites of equines (Pandit *et al.*, 2008). The major parasitic threats to equine health are Cyathostomins, *Parascaris equorum*, *Anoplocephala perfoliata*, and *Strongylus vulgaris* (Andersen *et al*., 2013). In addition, Takele and Sisay (2016) concluded; equines suffer from a number of gastrointestinal parasites. *Strongyles spp*, *T. strongylus, T. dontophors, P. equorum* and, Fasciola are the most important parasites exerting a significant economic impact wherever they are raised.

1.1.2 Veterinary importance of helminth parasites

Parasites pose large and real problems throughout the world today (Edward, 2006**a**). Parasitic infections constitute about 70-80% of animal diseases in Nigeria (Nicodemus, 2010). Parasitic infestations are the major cause of illness in donkeys (Muhammad *et al*., 2015; Burden and Getachew, 2016) commonly causing considerable mortality and morbidity (Sathiyamoorthy *et al*., 2016). Hence, helminth are the major constraints in donkeys present in significant amount in both rural and urban area despite of various differences in their feeding and management system. Various factors contribute in prevalence and abundance of gastrointestinal helminth including lack of proper care, awareness and effective deworming programs; which ultimately halts the growth of donkeys and their working ability (Rasool *et al*., 2016).

Animal parasites can affect their host in a number of ways: Firstly and foremost, parasites are likely to cause some type of physical trauma to the animal due to their migration within the body. Secondly, animal parasites excrete toxins in the body that may lead to a number of symptoms such as swelling, heart problems, digestive disorders, blurred vision, joint pain, and sleep problems (Edward, 2006**b**). Therefore parasitic helminthes are one of the most common factors that constrain the health and working performance of donkeys worldwide.

Parasites cause various degrees of damage depending on the species and number present, nutritional and the immune status of equids (Love *et al*., 1999). Parasitic helminth, especially strongyle nematodes, are commonly found in the large intestines of equines and can cause diseases with consequences ranging from ill-thrift to sudden death (Umur and Acici, 2009; Scantlebury *et al*., 2013). Official studies have shown that helminth are the major cause of death in donkeys in Ethiopia (Yilmaz, 2012). Abebew *et al*. (2011) showed that studies had shown that helminth in working donkeys were highly prevalent, infection intensities were very high and they are the major health problems of working donkeys in developing countries. In addition, Svendsen, (2015) mentioned that, lungworm (*Dictyocaulus arnfieldi*) can be present in large numbers in donkeys without the animals showing any signs. However, donkeys can pass on the infection to horses, which might suffer from lung problems resulting in coughing and discharge from the nostrils. Therefore, (Yoseph *et al.*, 2005; Getachew, 2006; Wako *et al*., 2016) concluded that, gastrointestinal parasites are the most serious health problem of donkeys in Africa, contributing to poor body condition, reduced power output, poor reproductive performance and short lifespan.

Donkeys are prone to infection with the same internal parasites or worms as horses, although 10 species of Small Strongyles are more prevalent in donkeys. The Lung worm is also more common in donkeys, although it rarely affects horses. Donkeys are more prone to the effects of resting Small Strongyles as encysted larvae in the hindgut wall and often develop diarrhoea, loss of condition and ill-thrift especially at the break of the season. They are also likely to harbour liver fluke if grazed on wet marshy areas with sheep and cattle, but exhibit few symptoms (Kohnke, 2011).

Nematodes belonging to the group of equine strongyles have a huge impact on equine health (Catalin *et al*., 2015). Strongylosis is one of the most important internal parasitic diseases of equines caused by nematodes of strongylidae family affecting more than 80% equids in the world (Khan *et al.*, 2015; Waqas *et al*., 2015). *Strongylus vulgaris,* one of the large *strongyle* is the most prevalent and pathogenic. Small strongyles exhibit mild symptoms of diarrhea and weight loss in the host whereas large strongyles show major pathogenesis (Khan *et al.*, 2015). Mortality rates caused by Small strongyles (*Cyathostomes*) can be as high as 40 – 70%. *Parascaris equorum* causes mild coughing and nasal discharge in the migratory phase. Heavy infections result in unthriftiness, a dull coat, poor growth in young stock, and lethargy. Younger animals can show signs of colic if a heavy roundworm burden causes a blockage of those parts of the digestive tract with a particularly narrow lumen (The Brooke, 2013). Therefore, gastrointestinal nematode infections constitute a threat to the health and welfare of donkeys worldwide (Scantlebury *et al*., 2013; Molla *et al*., 2015).

Helminth are present in all horse, pony, donkey and mule populations worldwide and are considered the major health problem of these species. Signs of worm infestation are quite variable, most commonly: poor growth, weight loss, poor coat, colic (mild to severe), tail rubbing, diarrhea (loose or soft manure), lethargy and lack of energy, anaemia (low red cells, pale or white gums) and sometimes death (Assis and Araújo, 2003; Canberra Equine hospital, 2013). These Worms are large strongyles (including *Strongylus vulgaris, Strongylus equinus and Strongylus edentates*), small strongyles (Cyathostomes), tapeworms (*Anoplocephala perfoliata*), roundworms (*Parascaris equorum*), bots (*Gasterophilus species*), pinworms (*Oxyuris equi*), thread worms (*Strongyloides westeri*) and lungworms (*Dictyocaulus arnfieldi*) (Canberra Equine hospital, 2013).

1.1.3. Economic importance of parasitic helminth

Presence of a disease in a livestock population can have several major effects, such as a reduction in efficiency of production through for example, death, reduced reproductive and growth rates (Ramsay *et al*., 1999). Parasitic nematodes of livestock have major economic impacts worldwide. Despite the impact of the diseases caused by these nematodes and the discovery of new therapeutic agents (anthelmintics), there has been relatively limited progress in the development of practical molecular tools to study the epidemiology of these nematodes (Florian *et al*., 2013).

Strongylosis is a prevalent disease and is an important health problem of the equines which is speculated to cause heavy economic losses through low performance and short life expectancy of working equines. Equines have crucial importance in the life system of developing countries especially in Africa, particularly for transportation (Bogale *et al*., 2012). Therefore, gastrointestinal helminth infection has great economic importance in equines and management was identified as a risk factor for the occurrence of helminth infection of equines (Belay *et al*., 2016).

1.2. Pathogenesis of parasitic helminth

One of the most common effects of parasitism is destruction of the host's tissue. This may be by mechanical action of parasites or their larvae migrating through or multiply in tissues or organs, or various organs of attachment. Destruction may be by pressure as a parasite grows larger or by blockage of ducts or vessels to produce infarction and oedema. Tissue damage may also be caused by the immunological response of the host, resulting in necrosis, dermatitis e.g. cercarial dermatitis or oedema in case of a scariasis and dictyocauliasis of the lung (Soulsby, 1982; Hansen and Perry, 1994).

The most significant pathology of large strongyles is caused by migration of the larval stage through the cranial mesenteric arteries and adjacent branches. Migration induces thrombus formation and thickening of arterial walls. Blockage of the blood supply to the intestine results in infarction and severe colic. The pathogenesis of strongylus encompasses severe enteropathy, verminous arteritis, damage of visceral organs, thrombosis leading to death and is mainly attributed to migrating larvae of parasites (Khan *et al.*, 2015). These are considered to be precursors of damage to organs of the digestive tract and severe disorders in enzymatic and hormonal processes (Assis and Araújo, 2003).

Small strongyles (Cyathostomes) have non-migratory life cycle cause verminous enteritis with profuse diarrhoea, colic, weight loss, dehydration, dullness depression and protein-losing enteropathy. If environmental conditions are not favourable for development (e.g. cold winter, dry season) larvae hypobiose (arrest development) and encyst in the large intestinal wall. The numbers of larvae in the large intestinal wall gradually increase over several months. When environmental conditions improve larval development is triggered. Mass emergence of encysted larvae from the gut wall causes significant damage to the large intestine (The Brooke, 2013; Gore *et al*., 2008).

In donkey populations in which all animals are administered anthelmintics on a regular basis, most harbour low burdens of parasitic Nematode infections and do not exhibit overt signs of disease. As in horses and ponies, the most common parasitic Nematodes are the *Cyathostomin species*. In some donkeys, encysted larvae build up in large numbers and can emerge synchronously to cause larval cyathostominosis. The latter is associated with one, or all, of the following: sudden onset weight loss, colic and, rarely in donkeys, diarrhea (Matthews and Burden, 2013). Moreover, Mcarthur *et al*. (2015) mentioned that; *Cyathostomins* are highly prevalent and potentially pathogenic parasitic nematodes found in the large intestine of horses and other equids worldwide.

1.3. Epidemiology of parasitic helminth

Although, the factors of the presence of parasitic diseases are numerous and mostly interactive, but the majority due to one or four factors. These are; the increase in the inter-active stages, change in host susceptibility, introduction of herd with high susceptibility and introduction of infection (Magzoub, 2002).

Although the environment is important, it includes surroundings and conditions either within the host or external to it that cause or allow disease transmission to occur. The environment may weaken the host and increase its susceptibility to disease or provide conditions that favour the survival of the agent. Environmental factors include aspects of climate (temperature, humidity, rainfall) as well as aspects of animal management (management of animals in a certain area of a country may result in high rates of disease that may not be seen in other areas) (Stevenson, 2005).

As far as host determinants, Putt *et al*. (1988) mentioned that, the main intrinsic determinants in the host which can influence the frequency of occurrence of infection and disease are species, breed, age and sex. Moreover, there are three major extrinsic determinants; the first two are climate and soils, which, by interacting in a variety of ways, affect the environment of the host, the agent, and the intermediate host or vector, if they are present. The third major factor is man, who uniquely among animals has the ability to modify both the environment in which he lives and the environment in which he keeps his animals.

Regardless of the age, sex and housing conditions most equines become periodically infected with parasites. Adults are usually infected with small and large strongyles and tapeworms. Young animals and foals can be also infected with *Parascaris equorum* and *Strongyloides westeri*. Prevalence of the parasitic infection depends on many factors including feeding conditions, and periodical deworming. Horses are particularly prone to parasitic infections during grazing on a pasture. If the pasture is not regularly cleaned from manure, it can easily become a reservoir of increasing number of infective larvae (Rajmund *et al*., 2015).

In working equids adults do not seem to develop such a strong immunity. Hard work and physiological stress may result in immune-compromise. Consequently, species such as *Parascaris equorum* may cause disease even in adults (The Brooke, 2013).

All common helminth parasites that affect horses also infect donkeys, so animals that co-graze can act as a source of infection for either species (Matthews and Burden, 2013; Burden and Getachew, 2016). The lungworm, *Dictyocaulus arnfieldi*, is problematical, particularly when donkeys co-graze with horses. Mature horses are not permissive hosts to the full life cycle of this parasite, but develop clinical signs on infection. In contrast, donkeys are permissive hosts without displaying overt clinical signs and act as a source of infection to co-grazing horse (Matthews and Burden, 2013). Moreover, Feye and Bekele (2016) concluded; the prevalence of lung worm (*Dictyocaulus arnifieldi*) infection rate was found higher in donkeys with poor body condition than medium and good body condition. Moreover, as compared to other literature reports, body condition, age and species of the animals were found to be the important risk factors associated with equine lung worm infection. Whereas, sex of the animals had no association with *Dictyocaulus arnfieldi* infestation in study animals. Animals of different species, age and sex group graze on communal pasture could favor the survival and might facilitated easy transmission of this parasite to uninfected animals. Hence, donkeys should graze in a separate pasture from horse and mule.

Large strongyles and cyathostomins are common in donkeys worldwide with *Strongylus vulgaris* causing significant disease in donkeys with poor anthelmintic treatment history. Donkeys are also susceptible to the flukes, *Fasciola hepatica* and *Fasciola gigantica* and may be an important reservoir host for both human and herbivore infections particularly in developing countries (Burden and Getachew, 2016).

A high prevalence of strongyle parasites may play great role in affecting the health and welfare of donkeys. High prevalence and egg count per gram of feces suggested that the presence of favorable conditions for survival, infection and perpetuation of this parasite of donkeys. Donkeys of all age groups, sex and body condition score were affected by Strongyle parasites. Severity of strongyle infection for working donkeys is significantly affected by age and deworming frequency while sex and body condition score may not reflect the degree of parasitic burden. Lack of effective veterinary service and poor awareness of animal welfare has exacerbated or aggravate prevalence of parasite in working donkeys (Wubie and Getaneh, 2015).

Worldwide, In Iraq, Wannas *et al*. (2012) found Gastro-intestinal Parasites in 100% of horses and donkeys. In donkeys, the prevalence of *Strongylidae*, *Parascaris equorum*, *Strongyloides westeri*, *Trichostrongylus axei, Oxyuris equi, Dictyocaulus arnfieldi, Cryptosporidium spp., Balantidium coli, Eimeria spp*. and *Entamoeba coli* were 57.14%, 32.14%, 28.57%, 17.85%, 17.85%, 17.85%, 19.64, 17.85%, 10.71% and 3.57%, respectively. Aypak and Burgu (2013) found the prevalence rate of *Trichostrongylus axei* (46%), *Habronema muscae* 56%, *Habronema majus*, (43.9%) and immature *Habronema sp.* (65%).

Parsani *et al*. (2013) found overall prevalence was 75.9%, and the gastrointestinal parasites prevalences were *Strongyloides westeri* (17.2%), *Parascaris equorum* (23.8%) *and Strongylus sp.* (55.3%). Whereas, in Pakistan, Waqas *et al*. (2014) found the overall prevalence of GIT parasites, was 55.66% and the prevalence of species were *strongylus, Trichostrongylus, Trichonema, Gastrodiscuss* and mixed infection was 28.33%, 9.66%, 6.33%, 6.33% and 5%, respectively.

In Pakistan, also Muhammad *et al*. (2015) found the overall prevalence of parasitic infestation was (78.33%). The gastrointestinal parasites were; *Strongylus sp*. (25%) was most common followed by *Parascaris sp*. (19.17%), *Strongyloides sp*. (14.17%), *Trichonema sp*. (14.12%), *Parafilaria multipapillosa* (8.33%), *Oxyuris sp*. (5.03%), *Dictyocaulus sp*. (3.33%) and *Triodontophorus sp*. (2.5%). Also, Rasool *et al*. (2016) found 81% of examined donkeys were having gastro-intestinal parasites and 10 gastrointestinal helminthes species were identified; *Ascaris* sp., *Strongylus sp*., *Trichomenna sp*., *Paranoplocephala sp*., *Triodontophorus sp*., *Anoplocephala sp*., *Gastrodiscus sp*., *Strongyloides sp*., *Oxyuris sp*. and *Dictyocaulus sp*. Majority of examined fecal samples showed mix infection and *Ascaris* nematode was the highest.

In Africa, Mezgebu *et al*. (2013) found that an overall prevalence of gastrointestinal parasites was 97.13% in donkeys, and the prevalence rates of species were 87.81%, 42.29%, 4.30%, 5.73%, 1.43%, 3.58% and 0.72% for *Strongyles, Parascaris equorum*, *Oxyuris equi*, *Fasciola*, *Tricuris*, *Gastrodiscus aegyptiacus and Gastrophilus intestinalis*, respectively. Also Berihun (2015) found the overall prevalence of major nematode parasites were Cyathostomum (81.7% and 100%), *Strongylus vulgaris* (68.3% and 97%), *Trichostrongylus axei* (62.2% and 92.5%), *Dictyocaulus arnfieldi* (21.2% and 49.4%), *Parascaris equorum* (15.9 % and 23.6%) and *Oxyuris equi* (7.6% and 10.4%) in intervention and control areas, respectively. Moreover, Tsegaye and Chala (2015) concluded that; a high prevalence of a wide range of species of gastro-intestinal helminth parasites that play a great role in confronting the health and welfare of donkeys. The overall prevalence of endoparasitic infection was 93.75%. The species of helminth found were; *Parascaris equorum* (20.5%), *Fasciola species* (15.36%), *Oxyuris equi* (15.36%) and *Dictyocaulus arnfieldi* (21.88%) and two genera (*Strongylus* and *Trichonema*), were encountered.

Recently, Wako *et al*. (2016) found the overall prevalence was (75%), and the highest prevalence was *strongyles* (33.9%) followed by (13.8%) *Ascaris*, (9.4%) *Strongyloid*, (8.1%) mixed infection, (5.2%) *Oxyuris*, (3.6%) *Fasciola* and (1%) *Trichuris*. In the same year, Belay *et al*. (2016) found the overall prevalence of gastrointestinal parasites in donkeys was 82.5%, and the species prevalences were 63.4%, 8.6%, 2.1% and 3.1% for *Strongyle spp*, *Parascaris equorum, Oxyuris equi* and *Anoplocephala spp*, respectively. In addition, Takele and Sisay (2016) found the overall prevalence of different gastrointestinal parasites was 86.51%. 3.87% of the donkeys harbored only one type of parasite (single infection), whereas 96.13% harbored two or more types of parasites (mixed infection). The parasites encountered were *Strongyles* (75.27%), *T. strongylus axei* (41.94%), *T. dontophorus* (43.01%), *P. equorum* (18.82%) and *Fasciola* (22.58%). Also Mohammed *et al*. (2016) stated that, gastrointestinal helminth parasites are endemic among the indigenous donkeys of Northeastern Nigeria, with an overall prevalence of gastrointestinal helminth 98.3%, of which 78.3%, 40.3%, and 17.5% were, *Strongyle, Parascaris equorum, and Oxyuris equi*, respectively**.**

Gebreyohans *et al*. (2017) concluded that the study indicated a high prevalence of a wide range of species of gastro-intestinal helminthic parasites that compromise the health and welfare of donkey. And they found the overall prevalence was 80.2% and the relative percentages were 52.0% *Strongyle spp*, 6.4% *Parascaris equorum*, 2.0% *Strongolides westeri*, 2.5% *Gastrodiscus aegyptiacus*, 2.7% *Anaplocephala Spp*, 2.2% *Fasciola* and 12.4% mixed parasites infection. And the mean EPG count of nematode parasites was found to be 925.25±662.82. Concerning severity of infection 57.6%, 26.4% and 32.5% of donkeys were infected severely, moderately and mildly, respectively.

Also, Kassa and Zeleke (2017) found the overall prevalence was found to be 72.33%, and the major gastro-intestinal parasites identified were *Strongyles spp.* (57.2%), *Parascaris equorum* (11.2%), *Strongyloides* spp. (10.4%), *Gastrodiscus aegypticus* (5.1%), *Oxyrus equi* (2.7%), *Fasciola* spp. (2.0%) and *Anoplocephala* spp. (2.6%). There was concurrent infection of donkeys with a maximum of two different gastro-intestinal parasites with prevalence of 33.48%.

In Sudan, gastrointestinal parasites, especially strongylids, are commonly present among donkeys. The prevalence of these nematodes among equines depends on many factors including environmental and breeding-related conditions (Romaniuk *et al*., 2007; Kornaś *et al*., 2008). Seri *et al*. (2004) found 70.1 % of the examined animals harbored a parasitical infection. Six nematode genera were encountered in donkeys, among them *Dictyocaulus arnfieldi* (70.5%) reported for the first time in Sudanese donkeys. *Strongylus sp.* (35.8%), Cyathostomes (36.7%), *Parascaris equorum* (10.7%), *Trichostrongylus axei* (12%), and *Strongyloides westeri* (3.4%). The incidence of infection with one species (53.2%) was found higher than that of mixed infection (46.7%).

According to severity of infection, 58.6% of the infected animals showed mild infection, while 21.9% and 19.5% of them showed moderate and severe infection, respectively. Cold season encouraged higher incidence of gastrointestinal nematodes (72.5%), more so than hot (69.3%) and rainy seasons (68.5%).

In Khartoum State, Sudan Najat (2006) concluded that; donkeys showed heavy parasite burden and all donkeys carried Nematode eggs and larvae without showing any ill health. Their egg counts varied and were significantly high but they seem to tolerate these worms without any ill effect. The most nematodes are *Strongylus vulgaris. S. equines, S. edentatus* and *Cyathostomes*. In South Darfur State, Sawsan (2009) found the prevalence of gastro-intestinal Nematodes was 37.48%. She observed the highest incidence of (55.79%) in January, while the lowest incidence (14.89%) in May. Severity of infection showed 81.25% for mild, 7.89% for moderate and 10.86% for severe infection. Arithmetic mean of egg per gram of faeces (epg) count was 750.14±1071.95 and the highest range reported was in April. While in North Darfur, Adam *et al*. (2013) found the overall prevalence with helminth parasites was 35.5%, and the most dominant genera of gastro-intestinal nematodes were *Strongylus spp, Cyathostomes spp, Trichostrongylus spp,* and *Strongyloides westeri.*

Recently, during their study on donkeys at Nyala town, South Darfur State, Sudan, Abdurhman *et al*. (2016) found that the overall prevalence rate of gastrointestinal parasites was 97.78%, and the parasites identified were *large strongyles* (84%), *small strongyles* (72%), *Habronema spp*. (40.2%), *Trichostrongylus axei* (30.4%), *Parascaris equorum* (18.5%), *Gastrodiscus aegyptiacus* (8.7%), *Anoplocephala perfoliata* (4.35%) and *Oxyuris equi* (1.1%).

1.4. Diagnosis

Studies and observations conducted in the last two decades have pinpointed helminth parasites as being a major health hazard, limiting the overall performance of quines (López-Olvera *et al*., 2006). To improve the welfare of equids it is important to prevent the onset of clinical disease. This can be achieved through reliable and quantifiable diagnosis of *strongyle* infections, which, in turn, allows the development of specific and targeted treatment, and prevention regimes (Elsheikha and Hallowell, 2014).

Diagnosis of parasitic infections depends on several factors, such as collection of the sample, transport of the sample to the laboratory, and method of laboratory evaluation. Diagnostic stages of most parasites can be detected in feces, blood, sputum, or skin scrapings. However, infections of immature parasites and latent and occult infections present a diagnostic challenge (William, 2001; Ambrosio and De Waal, 1990). The classical faecal examination techniques will remain the cornerstone of equine parasitology diagnosis and surveillance. However, more sensitive and specific techniques capable of diagnosing prepatent stages of *strongyle* infection would allow targeted and species-specific treatment, and prevent the unnecessary use of anthelmintic compounds in equines with low or no infection (Elsheikha and Hallowell, 2014). Fruitfully, (Ambrosio and De Waal, 1990) summarized; Laboratory diagnosis is a basic step in the evaluation of the disease process, at times confirming a presumptive diagnosis or providing evidence of an unsuspected agent of disease. Moreover, the effective control and treatment of parasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic protocol.

1.5. Control of equine helminth

Equine parasite control remains a complex and constant challenge for both owners and their veterinary advisers (Nielsen *et al.*, 2010). There are four major approaches to the control of helminth: ecological, which depend on shifting the local ecosystem to favor the hosts; chemical, in which drugs are used to treat, and in some cases prevent helminth infestations; genetic, where the aim is to breed livestock with enhanced resistance to important helminthes; and immunological, where the goal is safe and effective vaccines (Polley, 2010).

1.5.1. Treatment and drug resistance

Helminth are a diverse group of parasitic worms, encompassing Nematodes, Cestodes and Trematodes, and constitute a major health problem for human and animals in many parts of the world. In the absence of vaccines, control of these parasites is reliant on chemotherapy to ease symptoms and reduce transmission (Hatem, 2013). Therefore, control of parasites in donkeys must primarily focus on reducing the risk of infection, maintaining good health and targeting drug treatments carefully (Burden and Getachew, 2016).

Parasitic Nematodes of livestock are controlled mainly through anthelmintic treatment (Von Samson-Himmelstjerna, 2012; Florian, *et al*., 2013). Antiparasitic drugs have been used successfully to control parasitic diseases in animals for many years, as they are safe, cheap and effective against a broad spectrum of parasites (Vercruysse *et al*., 2007; Alsop, 2014).There are only three broad-spectrum anthelmintic groups available for treatment of grazing animals for the control of nematodes. Group 1; the benzimidazoles (BZ), group 2; the imidazothiazoles (Levamisole, LEV) and hydropyrimidines (Pyrantel / Morantel) and group 3; the Macrocyclic lactones (Ivermectins and Milbemycins, ML), have different mechanisms of action. The maintenance of the efficacy of existing anthelmintics is, therefore, essential for continuing animal productivity and welfare (Coles *et al.,* 2006; Kaplan and Nielsen, 2010). Currently, the only method available for determining if anthelmintics are effective on a farm is the faecal egg count reduction test (FECRT) in which faecal egg counts are measured both before and 14 days after treatment. Failure of drugs to achieve high levels of egg reduction following treatment indicates the presence of anthelmintic resistant parasites on that farm (Kaplan and Nielsen, 2010).

Most equine anthelmintics only kill luminal stages of cyathostomins, but the majority of all the worms can exist as larval forms encysted within the intestinal mucosa. Following treatment with an effective anthelmintic, these mucosal larval forms appear to quickly repopulate the lumen, mature, mate, and begin to produce eggs. The time required for eggs to reappear in the faeces following treatment is called the egg reappearance period (ERP) and this parameter differs for each drug. However, one cannot have egg reappearance if there never is egg disappearance. Thus, the existence of anthelmintic resistance on a farm will make the ERP parameter meaningless for a given drug.

Traditional control strategies mainly rely on the strategic application of anthelmintics, currently represented by three major drug classes: Benzimidazoles (BZ), the Tetrahydropyrimidine Pyrantel (PYR) and Macrocyclic Lactones (ML) (Von Samson-Himmelstjerna, 2012; The Brooke, 2013). Deworming increased the life span of donkeys by an average of 5 years (Yilmaz, 2012). At present there is no effective alternative to chemical control of parasitic helminth where livestock are grazed intensively. Resistance to anthelmintics has become a major problem in veterinary medicine, and threatens both agricultural income and animal welfare (Wolstenholme *et al*., 2004; Vercruysse *et al*., 2007; Hatem, 2013).

Development of variable degrees of resistance among different species of gastrointestinal nematodes has been reported for all the major groups of anthelmintic drugs. It has been observed that frequent usage of the same group of anthelmintic; use of anthelmintics in sub-optimal doses, prophylactic mass treatment of domestic animals and frequent and continuous use of a single drug have contributed to the widespread development of anthelmintic resistance in helminth (Hatem, 2013). Seasonal use of anthelmentics is the key to arrest the disease and overcome anthelmentic resistance. All stages of the life cycle of large strongyles are susceptible to benzimidazoles, Ivermectin and Moxidectin. In equids that are regularly de-wormed, *S. vulgaris* has been largely controlled (Khan *et al*., 2015).

Albendazole when used in the single dose expressed mean efficacy of (97.46%) and (98.55%) with the two doses administered at 14 days apart, while Ivermectin showed mean efficacy of (99.06%) against gastro-intestinal

Nematodes (Sawsan, 2009). For Cyathostomes, there are three effective drug classes: Benzimadazoles, Pyrantel and Macrocyclic lactones. Cyathostomin larvae have a low susceptibility to anthelmintics when in the hypobiosed state (The Brooke, 2013; Matthews and Burden, 2013).

Decades of intensive anthelmintic therapy have led to widespread anthelmintic resistance in cyathostomins across the world (Mcarthur *et al*., 2015; Salas *et al*., 2017). Unfortunately, deworming programs have become more and more haphazard, with poor client education and still easily available anthelmintics, often being sold from outlets with little or no accurate knowledge of how best to target deworm the equine population. The increasing practice of the use of ‘off label’ anthelmintics is also contributing to resistance development. The Nematodes are being exposed to sub therapeutic doses of the anthelmintics, thus providing ideal conditions for resistance development (Alsop, 2014). Therefore parasitic resistance to anthelmintic compounds has recently emerged as a significant concern in the equine industry (Eudy, 2007). Resistance to anthelmintic medication of equids strongyles is a worldwide phenomenon and for this reason systematic investigations of resistant parasite populations are necessary (Cernea *et al*., 2015). Development of resistance of several important equine parasites to most of the available anthelmintic drug classes has led to a reconsideration of parasite control strategies in many equine establishments. Routine prophylactic treatments based on simple calendar-based schemes are no longer reliable and veterinary equine clinicians are increasingly seeking advice and guidance on more sustainable approaches to equine parasite control (Nielsen *et al*., 2010).

Several factors can influence the rate at which anthelmintic resistance develops; high frequency of treatment is the most important. To reduce anthelmintic treatment frequency significantly, it is essential to examine the efficacy of the medication routinely for each drug class and to design a targeted control strategy, with management changes if necessary (The Brooke, 2013).

Also, monitor and evaluate de-worming strategies regularly using FEC, FEC reduction and by recording clinical disease caused by helminth. If resistant parasites are detected, withdraw the use of this anthelmintic.

During the past two decades anthelmintic resistance in equine parasites has been found in the group of small *strongyle spp* and *Parascaris equorum* (Von Samson-Himmelstjerna, 2012). Increasing levels of anthelmintic resistance reported worldwide in equine parasites have led to recommendations of constructing sustainable parasite control programmes based on systematic surveillance of parasite levels (Andersen *et al*., 2013).

Recently, Seyoum *et al*. (2017) forwarded the following recommendations: periodic epidemiological studies are essential to accurately establish the Nematode infection intensity and prevalence. It is important that farmers and veterinarians found a balance between achieving good parasite control and the sustainability of their control strategies to keep the effectiveness of available anthelmintic drugs. Creating awareness of the owners about resistance development of these drugs, avoidance of frequent dosing and under dosing, and also alternation with other anthelmintic drugs could be helpful. Further studies are needed to determine the anthelmintic resistance status of the different species of gastrointestinal parasites.

On different way, Scantlebury *et al*. (2013) mentioned that; there was extensive knowledge of plant-based treatments for gastrointestinal parasites in livestock in Ethiopia. In donkeys, Koso (*Hagenia abyssinica*), Grawa (*Vernonia amygdalina*), Enkoko (*Embelia shimperi*) and (mixed roots and leaves) were the most frequently named and or highest ranked plants with reported efficacy against gastrointestinal parasites. Further in vitro and in vivo investigation of these plants is now required to determine viable alternatives for the treatment and control of gastrointestinal parasites in Ethiopia.

1.5.2. Management

The prevention and control of parasitic Nematodes infection should take the management practices of equines and agro-ecology of an area into consideration by applying appropriate management and sanitary standard through strategic deworming (Molla *et al.,* 2015). Deworming twice, 3 weeks apart, with a broad-spectrum dewormer will help to reduce worm related colic and loss of condition (Kohnke, 2011). Sawsan (2009) recommended; donkeys need more attention to apply strategic programmes for controlling gastro-intestinal nematodes what makes donkeys more healthy and productive. Scheduled deworming programmes should be designed and applied on three occasions every year: January, July and October. Deworming protocols should start with Ivermectin drench followed by Albendazole suspension and finish with Ivermectin drench.

To control the burden of helminthes, Belay *et al*. (2016) recommended that; good management and awareness should be created regarding effective regular deworming, regular and strategic deworming programmes with efficacious anthelminthics should be carried out regularly, improved housing and feeding management system should be implemented to decrease the incidence of parasites in equines and the government should formulate and implement policies regarding management and health aspect of equines. Finally they recommended that, all newly introduced equines into the herd must be quarantined and properly screened and treated to prevent environmental contamination with helminth parasites.

The Brooke (2013) ; Corbett *et al*. (2014) mentioned; twice weekly removal of faecal material from pasture significantly reduced the number of strongyle eggs shed in faeces from groups of co-grazed donkeys. Use of this management control reduces the reliance and use of anthelmintic drugs, reducing the selection pressure towards cyathostomin resistance against these drugs. Finally, Berihun (2015); Rasool *et al.* (2016); Mohammed *et al*. (2016); Takele and Sisay (2016); Gebreyohans *et al*. (2017); Kassa and Zeleke (2017) concluded; the field veterinarian should aware the donkey owners on improving the housing and feeding management system and to providing sufficient food and shelter, minimizing overworking and extensive open grazing of their donkeys. More prevalence and efficient treatment and regular deworming program should be implemented using the available broad spectrum anthelmintics.

1.5.3. Vaccine trials

Control methods in which vaccines would have a central role provide attractive alternatives. However, while attenuated parasite vaccines have been successful, sub-unit vaccines are still rare. The advent of new techniques in molecular biology allows the elucidation of entire parasite genomes and the identification of individual genes. It is envisaged that a further understanding of parasite genes and the role of their products in parasite biology may lead to the identification of useful antigens, which could then be produced in recombinant systems. However, for this aim to be realized, continued investment in basic research on the complex interplay between parasite and host will be necessary (Vercruysse *et al*., 2007). Also they added at present, vaccines against parasitic diseases are relatively expensive when compared to the costs associated with drug treatment. The incentive to use vaccines is, in some cases, related to a lack of efficacy in the parasitic drug. Indeed, efforts towards vaccine development should be pursued intensively while drug based infection control persists; it is pointless to wait until effective control is lost. Many vaccines may find their greatest and most immediate application in integrated control strategies. The synergies offered by a combination of vaccines and parasiticides should be thoroughly explored, as this approach may lead to a substantial reduction in the use of parasiticides.

1.6. Donkeys

There are approximately 100 million working equids in developing countries worldwide (FAOSTAT, 2012). The equine population of the world is

98.3 million (40 million donkeys, 15 million mules, 43.3 million horses). In the distribution pattern, 98% of all donkeys, 97% of all mules, and 60% of all horses are found in developing countries. The number of equines in Africa is in the range of 17.6 million, comprising 11.6 million donkeys, 2.3 million mules and 3.7 million horses (Takele and Nibret, 2013). Working donkeys, horses and mules make up approximately 112 million of the global livestock population in less developed countries (Valette, 2014; Valette, 2015).

1.6.1. Definition

The donkey or ass (*Equus africanus asinus*) is a domesticated member of the horse family, Equidae. The wild ancestor of the donkey is the African wild ass, *E. africanus*. The donkey has been used as a working animal for at least 5000 years (Grubb, 2005; Kugler *et al*., 2008). There are two distinct species of wild donkey; the Asiatic branch of the species (*Equus hemionus*) came from an area stretching from the Red Sea to Northern India and Tibet. The African branch of the species (*Equus africanus*) was found in North Africa between the Mediterranean coast and the Sahara Desert to the south of the Red Sea. The modern domesticated donkey, on the other hand, is descended from African wild ass ancestors, of which there were two separate species: the Nubian wild ass (*Equus africanus africanus*) and the Somali wild ass (*Equus africanus somaliensis*). Donkeys were first domesticated around 6,000 years ago in North Africa for meat and milk (Svendsen, 2015).They enjoy browsing on woody, thick-stemmed plants, branches, bark, thistles, blackberry and other coarse grasses and plants which are left by horses and cattle (Kohnke, 2011).

Equus asinus individuals typically have narrow, concave and slightly flexible hoofs which are suitable for rocky areas rather than flat desert or sand dunes. The normal lifespan of a donkey in good health can exceed 50years, but their lives are often shortened by poor management and parasite infestation. They are characterized by toughness and can survive in arid areas and on poor quality of food. They are obedient, docile, and easy to manage, can be handled even by women and children (Yilmaz, 2012). Donkeys reach maturity around four years of age, with maximum weights being reached at about six years of age. In Africa, donkeys generally weigh about 120-180 kg. Naturally, good management affects the speed of growth and final body characteristics. With good care, donkeys can have a working life of 12-15 years, and they can live even longer (Oudman, 2004). Therefore, donkeys still play an important role in Africa, despite mechanization in the 21st century. This is due to a number of factors including the resilience and adaptability of donkeys to the harsh conditions in most parts of the continent; their ability to access narrow paths between farmlands; and their relative resistance to endemic diseases like trypanosomiasis compared to oxen (Ogola *et al*., 2014).

Although they have sometimes been considered as animals of ridicule or low status, they have excellent reputations as easily trainable and very dependable work animals. Furthermore can easily be managed by children. In addition, Aganga *et al.* (2000); Burden (2012) mentioned; donkeys and mules have evolved to survive on highly fibrous forages that they would naturally graze and browse on whilst walking many miles per day over challenging terrain. The most appropriate source of energy and dietary fibre for fit donkeys with good dentition are cereal straws (cereal stalks remaining after the grain has been harvested). They may require dietary supplementation with hay or haylage during the winter or when pregnant, lactating or growing in order to supply extra energy.

1.6.2. Classification

Donkeys belong to the order of odd-toed Ungulates (Perissodactyla), sub-order Horse-like (Hippomorpha). They belong to the Horse-Family (Equidae). This family includes the genus Horse (Equus) with five under-genera: Wild Horse (*Equus caballus*, or. *Equus przewalski*), Wild Ass (*Equus hemonius*), Ass (*Equus asinus*), Zebra (Hippotigris), and Grevyzebra (*Equus grevyi*) (Kugler *et al*., 2008). They were divided into three subspecies: North African Wild Ass (*Equus asinus atlanticus*) Nubian Wild Ass (*Equus asinus africanus*), and Somali

Wild Ass (*Equus asinus somalicus*). Moreover**,** Yilmaz (2012) classified donkeys as follows; Kingdom: Animale, Phylum: Chordata, Class: Mammalia, Order: Perissodactyla, Family: Equidae, Genus: Equus, Subgenus: Asinus Species *E. asinus*.

1.6.3. Socio - economic importance of donkeys

Working donkeys play a fundamental role in human livelihoods through their direct and indirect contributions to financial, human and social capital in particular. They are important in communities and households socio-cultural lives, as they are often used in celebrations and in supporting households in need by being lent and shared between families. Working donkeys are multipurpose: they provide draught and load-bearing power, as well as outputs including manure and sometimes milk, meat and hides. Working animals create synergy in nutrient cycles, farming and marketing systems by enabling farmers and traders to transport harvests, market products, fodder and water for other livestock (FAO, 2011).

As donkeys are working animals, they have good traction capability. In addition, they are cheap to buy and economical to use by small-scale farmers. Therefore, donkeys are used by human for draught, pack, and ridden work, milking, breeding, and sometimes eating (Yilmaz, 2012). Donkeys are docile rural equids, traditionally used as working animals (Ragona *et al*., 2016). For these and other reasons, donkeys remain of crucial economic importance in many undeveloped and developing countries (Yilmaz, 2012). Hence, donkeys are amongst the oldest companion of man. They are very hardy animals and can work incessantly with little rest and on poor forage (Kataria and Kataria, 2010). The donkey's role socially, culturally and economically varies widely depending upon the communities in which it lives and works (The Donkey Sanctuary, 2017).

Domestic working donkeys, horses and mules are primarily used to provide pack and cart services exclusively for transportation of household members and or their goods and for helping families with household chores and labour (e.g. fetching water and firewood). Therefore, working equids are a source of financial stability for families and they relieve human burdens, for women and children in particular (Knottenbelt and Deceim, 2015). In addition, working donkeys, horses and mules are used to earn an income for their owners in a number of industries (e.g. agriculture, tourism, public transport, construction, transport of goods) either directly (payment for service) or indirectly (support to owner's income generation activities). Therefore, working donkeys, horses and mules generate direct income in a number of industries in both urban and rural settings (Madure, 2014; Valette, 2015).

Donkeys need little attention and small quantities of food to provide sustained work on poor forage with little rest. As a result, they are considered to be excellent pack animals for transporting heavy loads in the hills, desert and plain areas in countries such as India, Egypt, Sudan, Somalia, Persia and China. In tropical countries such as India, they are the cheapest and easiest means of transport, suiting needs of washer man, potters, house builders and brick manufactures (Mwenya and Tandkeib, 2004). Therefore, donkeys play a crucial role in transport services and economy within both rural and urban communities (Pearson *et al*., 2000; Kendagor and Njoroge, 2014; Asmamaw *et al*., 2014).

It is estimated that 50 per cent of the energy required for agricultural production in the world is derived from animals, and the donkey is a major contributor to this need (Parsani *et al*., 2013). Nevertheless, Donkeys are an undervalued power source in a large part of the world. Their potential to work is very high and their contribution to any household or even national economy is considerable. Generally the buying and selling price of donkeys is far below their true value, which should be calculated on the basis of the work they give over the 14 years they are able to work, if well cared for. If a donkey works six hours a day, four days a week over that many years, it will have given about 15,000 hours of work. The low price of donkeys, therefore, is a reflection of distorted perceptions of their role. This situation is changing. In Zambia, for instance, donkeys are now selling for the same price as cattle (Oudman, 2004). In Sudan (especially West Kordofan), the price of faster donkey which used for riding and transport is now approximately double the price of the local cow (researcher observations).

Donkeys are particularly useful in reducing the burden of women in daily chores such as transporting water, firewood and farm produce, and can also provide an income for women through enabling access to markets for surplus produce (FAO, 2011). The great majority of donkeys in the world (probably over 95%) are kept specifically for work. In most countries, donkeys can be owned and used by either men or women. Children are frequently given responsibility for working with donkeys. The donkey population in Pakistan is over 4 million heads. There are over 1,000 donkey carts in the city of Faisalabad, working all day, every day fetching one of life’s most essential ingredients and water (Muhammad *et al*., 2015).

Today, in many parts of Italy, donkey is rediscovered for the potentiality and adaptability in different environments that can still have. The presence of donkey becomes once again useful inside farms, first of all, because of its milk production, milk that is similar to that of woman’s breast milk and, also, for the use of this milk in the industry of cosmetics (Karatosidi *et al*., 2013). Donkey milk was characterized by the high lactose content, low fat, higher levels of unsaturated fatty acids compared to ruminant milk. Unsaturated fatty acids and omega 3 fatty acids in particular have become known for their beneficial health effect. Milk quality characteristics support its use in infants and children affected by food allergy to bovine milk proteins, and also in adults with dyslipidemias and in the prevention of cardiovascular disease (Ragona *et al*., 2016). It is written that Cleopatra, queen of ancient Egypt, used donkey’s milk in order to preserve the beauty of her skin. It seems also that Poppea, second wife of Emperor Neron, did the same thing. Donkey’s milk is still being used for the production of soaps and moisturizers and it is much in demand by manufacturers of cosmetics. The father of medicine, Hippocrates, prescribed this milk for many diseases like liver disease, edemas, nose bleeding, poisoning, infectious diseases, healing of wounds and fevers. It was used until the early twenties as a substitute for woman's breast milk because of its chemical composition that is considered closer to that of humans and, therefore, it is being used mostly for the feeding of children in the infant period, ensuring good growth and normal mental and physical development (Karatosidi *et al*., 2013).

Traditional consumption of donkey meat is particularly widespread in Italy. In particular, in the valley of Padania, in recent years, the slaughter of old horses and donkeys helped inhabitants to add proteins to their daily meals that were mainly based on grains and vegetables (Karatosidi *et al*., 2013). Also, according to (DPIR, 2016) there is a tradition in Asian and European countries of farming donkeys for milk and meat. In some Asian countries donkeys are grown for hides which are then processed into gelatin and used in edible preparations, traditional Chinese medicine and cosmetics. Therefore, most notable within the last two years has been the emergence of large-scale global trading in donkey skins, with estimates of a minimum of 1.8 million donkey hides being traded per year as in **plate 1.1.** In turn, global demand has been conservatively estimated to be up to 4 million (China daily, 2016), with some sources reporting upper limits of demand in China to be 10 million skins per annum (Zhu Wenqian, 2015). Donkey skins are used to produce a traditional Chinese medicine (TCM) called ejiao, otherwise known as Colla corii asini or 'donkey hide glue, which is based upon extracts of donkey gelatin in the hide mixed with herbs and other ingredients, often to form a gelatinous bar, pill or tonic. Ejiao is a medicine with ancient roots and has been promoted as a product worthy of emperors. Wide-ranging health benefits are claimed for the products, including anti-ageing properties, an increase in libido and a reduction in reproductive-organ disease in women (Wang *et al*., 2014).

Therefore, donkey faces again a glory due to the diffusion of the culture for the protection of the environment, the rediscovery of old traditions, the need for more direct contact with nature, by producing new elements for alternative medicine for the treatment and prevention of typical human diseases (depression, loneliness, etc.) of a deep technological and materialistic society. In the last few years, donkeys have become the principal "actor" of original and innovative processes with the idea to form an important area in the global zootechnical backstage (Karatosidi *et al*., 2013). **Figure 1.1** illustrates; working equines support people's livelihoods in a wide range of sectors including agriculture, construction, tourism, mining, and public transport. It is estimated that working equine animals help approximately 600 million people globally, very often in the poor and marginalized communities (Valette, 2015).

Plate 1.1: Donkey hides drying in the sun as part of the production process of ejiao (Source: The Donkey Sanctuary, 2017**)**

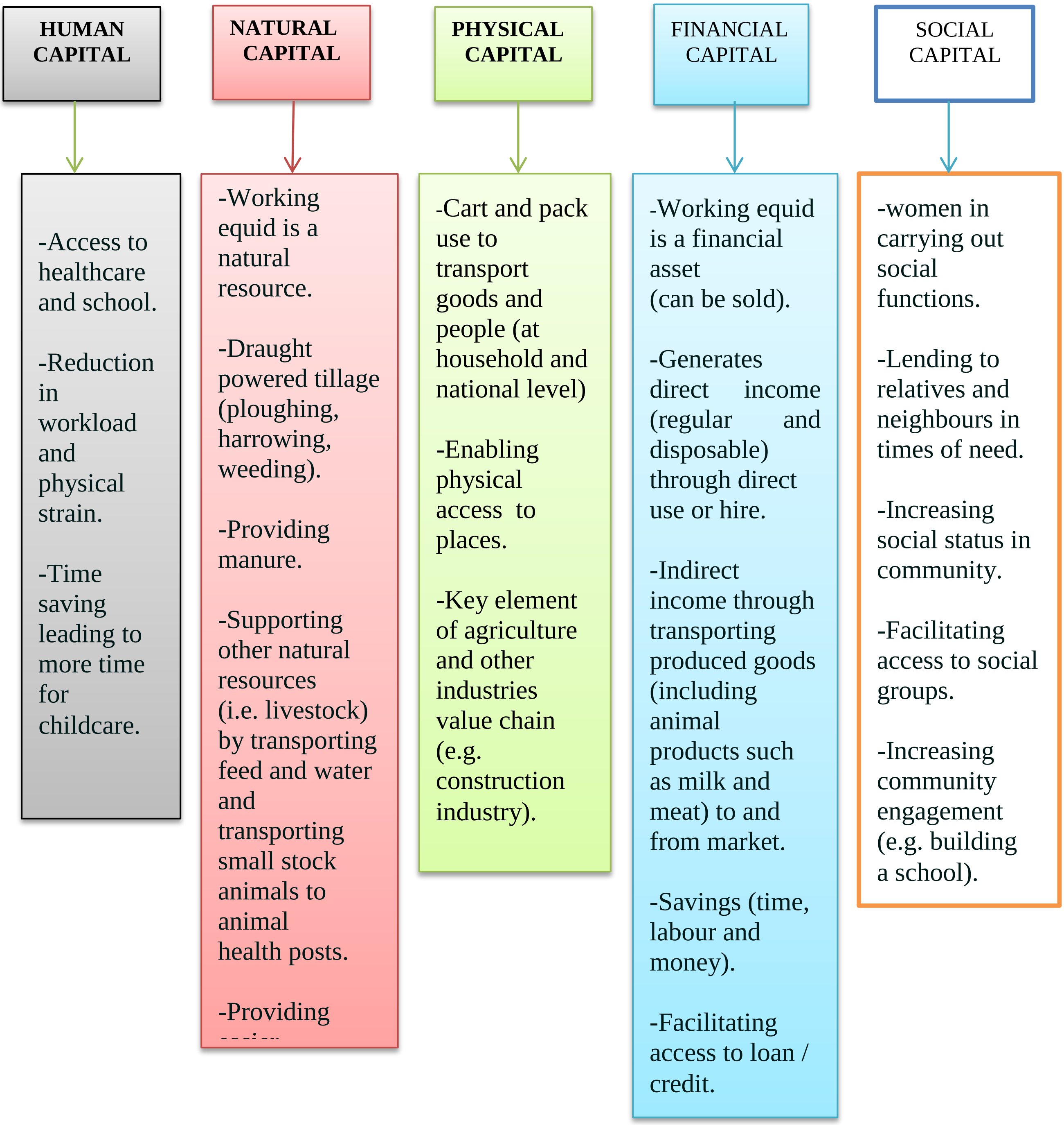


Figure 1.1: How working equine animals contribute to people's livelihoods

Source (Valette, 2015)

1.6.4. Health problems of donkeys

The main problems facing donkeys were wounds and injuries, helminthiasis, fly attacks and sudden deaths (Chemonges *et al*., 1997).

In across-sectional study was conducted by Ashinde *et al*. (2017), the prevalence of wound, lameness, skin problem, other illness signs, eye problem, dental problem and change on visible mucous membrane were found in 47.7, 38.8 36.2, 14.3, 9.4, 7.6 and 6.3%, respectively. Furthermore, 63.2% of donkey users were not aware of common animal welfare freedoms. The health and welfare problems of cart pulling donkeys in the study area were created and complicated with multiple influential factors. The whole community should participate on awareness creation, introduction of improved design of harnesses and carts, also training on animal welfare to owners should be given to reduce health and welfare problems on donkeys. Other similar studies conducted by (Moreda *et al*., 2015; Assefa *et al*., 2017).

In another study, Abate (2017) concluded; working donkeys have been suffering from high incidence of wound followed by musculo-skeletal disease, infectious disease, nutritional, reproductive and parasitic disease in the same order of importance. Most of these problems affected adult working donkeys and were related to poor donkey management system practiced by the donkey owners. Particularly the high incidence of wound on the long axis of the animal is associated with improper harnessing and over loading. Generally adult animals with poor body condition were more affected with different kinds of health problems.

Chapter Two

General materials and methods

2.1. Study area description

Ghebaish and Alfula localities of West Kordofan State were selected to study the prevalence rates of helminthic infestation, its associated risk factors and to investigate prophylactic measurements for the control of helminth infestation in draught donkeys. West Kordofan is one of the 18 states of Sudan. It has an area of 111 373 km² and an estimated population of approximately 1 320 405. Alfula town is the capital of the state. The state of West Kordofan is located in the South-Western part of the region of Kordofan in Sudan which lies between latitude 11º - 20º N longitude 32º. 22″ - 30º. 27″ E **(Appendices 9 and 10)**. The Southern part characterized by heavy rainfall and vegetation with heavy clay soils, and the Northern part is a medium-range rain and prevailed sandy soil (West Kordofan University, 2017). The major activities of the population are agriculture and livestock grazing, and rain fed farming of millet, sorghum, groundnut and water melon. Almost all population practicing these activities depends absolutely on donkeys as a mean of transportation of people and serving of all requirements of agricultural and livestock activities in the area.

2.2. Study type and design

This is a cross-sectional study conducted in West Kordofan (Ghebaish and Alfula localities), the center of the western regions of the Sudan to assess the prevalence rates of helminthic infestation, its associated risk factors and to investigate prophylactic measurements for the control of helminth infestation in draught donkeys in West Kordofan State. Samples were collected from entire two cities and surrounding villages.

2.3. Sample size

A total number of 1200 donkeys were randomly selected from Ghebaish (600 animals) and Alfula (600 animals) towns (and some of their surrounding villages) which were the major sites for draught donkeys. Donkeys were randomly included in the study regardless of sex, age and health status.

The sample size was calculated according to Stevenson (2005) as follow:

n= $(1-(\alpha)^{1/D}) \times (N-(D-1) \times 1/2)$ where:

N: the population size

α: 1 - confidence level (usually a = 0.05)

D: the estimated minimum number of diseased animals in the group (population size ×the minimum expected prevalence)

2.4. Sample collection

A total of (1200) faecal and (600) blood samples were randomly collected from draught donkeys during three major annual seasons: wet hot, dry cool and dry hot, to screen for helminth infections and to evaluate the epidemiologic burden of these parasites among draught donkeys in one of the highest donkey population region of Sudan. Blood samples were taken to assess the anaemic status of the studied donkeys using Pack Cell Volume (PCV). Each animal was sampled once in a season. A number of 200 faecal samples and 100 blood samples were collected from each locality in every season.

2.5. Donkeys' owners information

The annexed questionnaire had been developed, tested and applied in the study area aiming to assess the risk factors which influencing the helminth infection in draught donkeys in West Kordofan State, so as to detect the real helminth problems. About 600 donkey owners were selected and subjected to the questionnaire using convenience sampling (100 owners per season and locality).

2.6. Clinical trial:

For future scope in order to implement strategies for prevention, an additional clinical trial was performed. Moderate to heavy infected donkeys were further selected and categorized in to four groups, then treated using different treatment schedules.

2.6.1. Trial site and animals treated

Ghebaish locality was chosen for the experiment as having easily accessed animals, facilities and suitable management for the performance of this type of experiment. Moreover, the treated animals were left on field with their owners, on their normal environment and management system. A round (40) working donkeys of different ages and sexes were selected from infected animals on the basis of confirmed infection and willingness of owners. The selected animals were grouped in to (4) groups for treatments. Animals were faecally sampled and the egg was counted for each individual animal. Each group of donkeys was treated using one type of anthelmintic.

2.7. Statistical analysis

The data were analyzed using statistical package for social studies (SPSS) software version (SPSS version 16). Descriptive statistics including frequency and cross tabulation, Correlation, and frequencies were used to identify the risk factors. Chi-Square test was used to test the null hypothesis when there was no significant difference in exposure incidence in different areas in study sites. P value less than 0.05 using 95% confidence level was considered statistically significant.

Chapter Three

Seasonal prevalence of helminth infestations in donkeys

3.1. Introduction

Gastrointestinal (GIT) nematode infection was highly prevalent in donkeys and young age group where as sex and body condition was not significantly associated with prevalence of GIT nematode. Stronyle and *Parascaris equorum* was the nematode parasites affecting donkeys and horses in Hawassa town, and Strongyle had the highest relative prevalence than Parascaris (Tesfu *et al.*, 2014).

Sudan has a large and species-diverse array of domestic animals. Estimates by the Food and Agriculture Organization (FAO) puts the Republic of Sudan, since its partition with the Republic of South Sudan in 2011 as having the third largest total livestock population of all African countries. The country ranks first to third in Africa in cattle, sheep, goat and camel numbers, third in poultry and fifth in donkeys (FAO, 2014).

In Sudan Donkeys were used either as pack animals (8.9%) or most dominantly for pulling carts (91.1%) (Angara, 2011). Hence the importance of donkeys in the Sudan is unequivocal. The animal provides support and transport at a low cost for urban and rural areas (Abdurhman *et al*., 2016).

Parasitic helminth continue to be the major constraints affecting the health and working performance of donkeys worldwide. They cause various degrees of damage depending on the species and nutritional and the immune status of the donkey. They decrease the performance and productivity through reduction of body weight or retarded weight gain and even death in acute cases. Hence, further studies are recommended on the impact of parasitic infections on the health status, working efficiency, reproductive efficiency, draft ability, and longevity of donkeys (Mohammed *et al*., 2016). Many studies from some parts of Sudan highlighted the significance of donkey in livelihood and prevalence rates of helminthes between donkeys. However there were no studies has been conducted in West Kordofan about the link between donkeys and helminth infections. Therefore, this survey about helminth infection status was done in West Kordofan State to fulfill the following objectives:

1- To study the prevalence rates of helminth infestation in draught donkeys in the study area.
2- To identify the pathogenic helminth parasites among draught donkeys in the study area.
3- To make comparison of the findings between the two localities in the study area.

3.2. Materials and methods

3.2.1. Faecal samples

3.2.1.1. Faecal samples collection and examination

A total of 1200 faecal samples were collected from Ghebaish and Alfula localities, West Kordofan State during the three main seasons (Wet hot, dry cool and dry hot). From each animal a faecal specimen was taken directly from the rectum and placed in faecal sample container (airtight and labeled container). Each batch of collected specimens was transferred to the Parasitology Laboratory for microscopic investigation within 2 hours (in cold chain facility). Drops of 10% formaldehyde were added to the sample container for the specimens expected to be examined within extended time (1-2hours). Samples were examined in two centers; at the Parasitology Laboratory, College of Veterinary Medicine, West Kordofan University in Ghebaish and at the research laboratory in Alfula. Specimens were initially examined for morphological changes, odour and colour. Modified McMaster slide technique was used to count egg per gram (epg) faeces to assess the worm burden.

3.2.1.2. Intensity of helminth infection

The severity of infection which obtained from the number of eggs per gram of faeces was determined according to Soulsby (1982), as follow:

500 eggs/gram of faeces = mild infection

800 – 1000 eggs/gram of faeces = moderate infection

1500 – 2000 eggs/gram of faeces = severe infection

3.2.1.3. Technique used for identification and egg count

The egg count was performed adopting McMaster technique, which was described by William (2001).

Procedure:

Three grams of faeces were taken from each collected fresh sample and mixed with 42 ml of fecal flotation solution Sodium Chloride (400 g NaCl in 1 litre tap water = specific gravity 1.18 - 1.2), then the faecal mixture was poured through tea strainer (sieve) **(Appendix 1)**. The solution was removed from the strainer as much as possible by pressing on the material. The strained material was poured into a 15ml centrifuge tube and centrifuged at 1,500 rpm for 2 minutes. With a pipette or syringe, both of the chambers in the McMaster slide were loaded with the strained solution. The slide was allowed to sit for a few minutes for the flotation process, then using a microscope; the slides were examined, focusing on the top layer so that the grid lines are in focus. The counts for each type of parasite egg were recorded in both chambers inside the gridlines (using the battlement method). After reading, the slide was washed thoroughly to be ready for the second use. The number of eggs per gram of feces was calculated as follows:

The chamber counts the eggs in 0.15 ml was used. Consequently, the total number of eggs counted was found in 0.3 ml. Therefore, the number of eggs found in the two chambers was multiplied by 100, and then divided by 2 to convert the number into eggs per one gram of feces (Or just the number of eggs in the two chambers multiplied by 50).

The mathematics: *0.15 ml is 1/300 of 45 ml (42 ml water and 3 gm. feces) so the number of eggs in 0.15 ml X 100 is equal to 1/3 of the total number of eggs in the original 3 grams and thus equal to eggs per gram (EPG).*

3.2.2. Blood samples

A total of 600 blood samples were taken (100 samples per season and region). About 3 ml of blood were withdrawn from the jugular vein using sterile syringes. The blood was immediately transferred to heparinized containers (tubes), and then transferred to the Laboratories for hematological examination.

The Packed cell volume assessment

Blood were taken from the heparinized tubes (brought from the field) in a heparinized capillary tube to determine packed cell volume (PCV). The vacant end was sealed with sealant. The capillary tubes were centrifuged at 1500 rpm for 5 minutes then the value of PCV was determined using a microhaematocrite reader according to Abebew *et al*. (2011).

3.3. Results

3.3.1. Faecal samples examination

A total of (1200) faecal samples were collected from the study area and examined using Modified McMaster slide technique to diagnose and assess the prevalence of helminth in draught donkeys of different sexes and ages. Most of the faeces were found normal and few were soft and diarrheatic. Out of 1200 studied animals, 459 animals were found positive with 9 different pathogenic parasites, giving an overall prevalence rate 459/1200 (38.25%). However, the prevalence rates of parasitic infections in Ghebaish and Alfula were 243/1200 (20.25%) and 216/1200 (18%), respectively. Moreover, the prevalence rate of infection in Ghebaish was 243/600 (40.50%), and in Alfula was 216/600 (36%).

The major nine pathogenic parasites were identified in this study included: *Strongylus spp, Dictyocaulus spp, Strongyloids spp, Cyathostomes, Oxyuris spp, Parascaris spp, Anoplocephla spp, Paranoplocephla, spp* and *Trichostrongylus spp.* The great majority of the infected animals were found infected with *strongylus* representing 275/459 (59.91%) followed by mixed infection, *Dictyocaulus, Strongyloids, Cyathostomes, Oxyuris, Parascaris, Anoplocephla, Paranoplocephla* and *Trichostrongylus* constituting 77/459 (16.78%), 30/459

(6.54%), 23/459 (5.01%),16/459 (3.49%), 16/459 (3.49%), 12/459 (2.61%), 5/459 (1.09%), 3/459 (0.65%) and 2/459 (0.44%), respectively as in **Fig 3.3.2**.

The majority of helminth infections were found in Ghebaish locality represented 243/459 (53%), whereas, in Alfula locality the parasites were 216 /459 (47%). The main infections in Ghebaish were *strongylus*, mixed infection, Cyathostomes and Dictyocaulus with percentages; 157/243 (64.60%) and 40/243 (16.46%), 13/243 (05.35%) and 9/243 (03.70%), respectively. And the main detected parasites in Alfula were; *Strongylus*, mixed infection, *Dictyocaulus*, *Strongyloids* with percentages of 118/216 (54.63%), 37/216 (17.13%), 21/216 (9.72%), 16/216 (7.41%), correspondingly, which were constitute the main infections in Alfula as illustrated in **Table 3.3.1.**

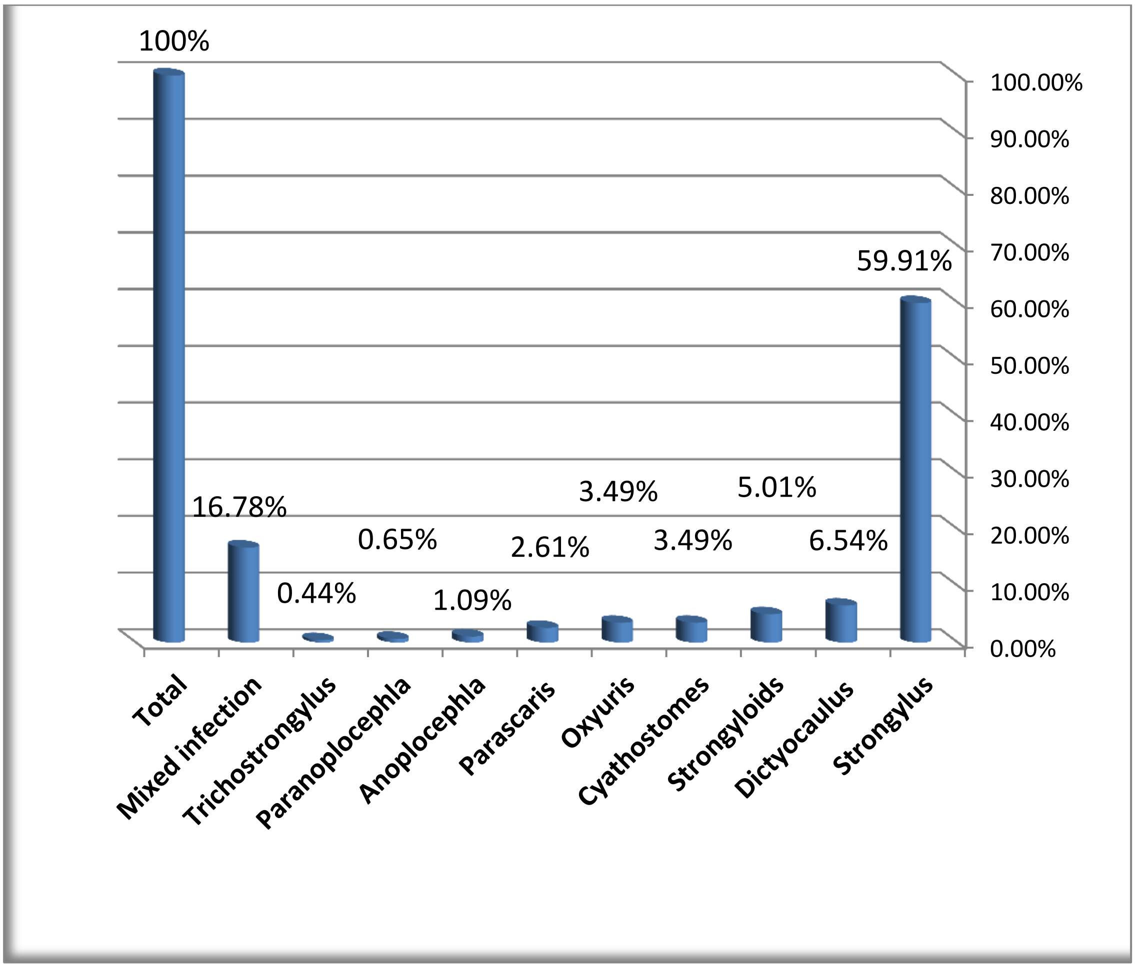


Figure 3.3.2: Description of the proportions of helminth species in the study area

Table 3.3.1: Epidemiology of helminth parasites in Ghebaish and Alfula localities

Parasites	Locality					
	Ghebaish		Alfula		Total	
	Frequency	%	Frequency	%	Frequency	%
Strongylus	157	64.60	118	54.63	275	59.91
Dictyocaulus	9	03.70	21	09.72	30	06.54
Strongyloids	7	02.88	16	07.41	23	05.01
Cyathostomes	13	05.35	3	01.39	16	03.49
Oxyuris	6	02.47	10	04.63	16	03.49
Parascaris	6	02.47	6	02.78	12	02.61
Anoplocephla	4	01.65	1	00.46	5	01.09
Paranoplocephla	1	00.41	2	00.93	3	00.65
Trichostrongylus	0	00.00	2	00.93	2	00.44
Mixed infection	40	16.46	37	17.13	77	16.78
Total	243		216		459	
	(53%)	100.00	(47%)	100.00	(100%)	100.00

With regard to the seasonal distribution of positive donkey samples, most of infections were found during dry hot season representing 237/459 (51.63%) followed by wet hot and dry cool constituting 114/459 (24.84%) and 108/459 (23.53%), correspondingly as in **Fig 3.3.3.** *Strongylus spp* was found predominant in the three seasons, high in the dry hot followed by dry cool and then wet hot season. Cyathostomes were appeared only during wet season, whereas Trichostrongylus was found in the dry hot season only.

In dry hot season, the main parasites were *Strongylus* followed by mixed infection, *Strongyloids* and *Parascaris* representing 167/237 (70.46%), 38/237 (16.03%), 15/237 (6.33%) and 8/237 (03.38% respectively. In wet hot season, the high percentages were 46/114 (40.35%), 21/114 (18.42%), 16/114 (14.04%), 13/114 (11.40%) and 8/114 (7.02%) for *Strongylus*, mixed infection, *Cyathostomes*, *Dictyocaulus* and *Oxyuris*, respectively. In dry cool season the main parasites were found as the following; *Strongylus*, mixed infection, *Dictyocaulus* and *Oxyuris* with the percentages 62/108 (57.40%), 18/108 (16.67%), 13/108 (12.04%) and 7/108 (6.48%), respectively as in **Table 3.3.2.**

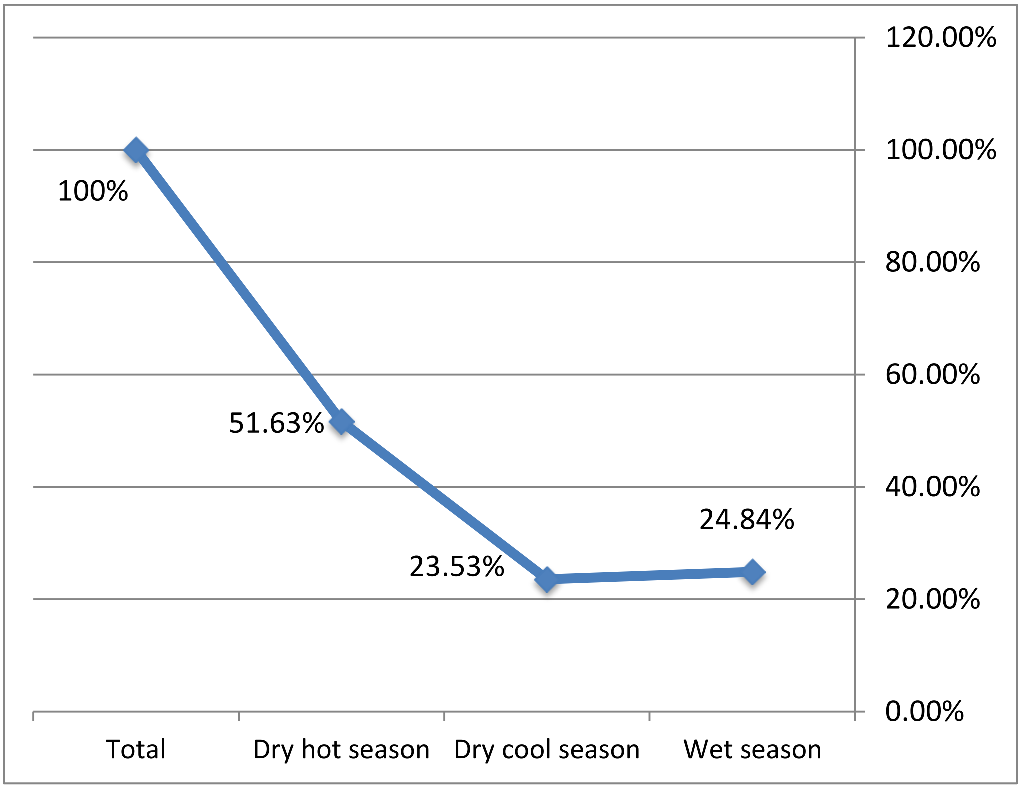


Figure 3.3.3: The seasonal description of the infected draught donkeys in the study area

Table 3.3.2: Distribution of helminth parasites by the three seasons

Parasites	Season						Total
	wet		dry cool		dry hot		
	Frequency	%	Frequency	%	Frequency	%	
Strongylus	46	40.35	62	57.40	167	70.46	275
Dictyocaulus	13	11.40	13	12.04	4	01.69	30
Strongyloids	5	04.39	3	02.78	15	06.33	23
Cyathostomes	16	14.04	0	00.00	0	00.00	16
Oxyuris	8	07.02	7	06.48	1	00.42	16
Parascaris	3	02.63	1	00.93	8	03.38	12
Anoplocephla	0	00.00	3	02.78	2	00.84	5
Paranoplocephla	2	01.75	1	00.93	0	00.00	3
Trichostrongylus	0	00.00	0	00.00	2	00.84	2
Mixed infection	21	18.42	18	16.67	38	16.03	77
Total	114 (24.84%)	100	108 (23.53%)	100	237 (51.63%)	100	459 (100%)

Therefore, the prevalence of *Strongylus spp* and mixed infection were found to be significantly higher in the two regions and mainly in Ghebaish during the dry hot season.

The donkey ages were available for 600 animals screened. Out of 600 animals, 231/600 (38.50%) were found to be infected with different parasites types. The majority of infected animals were found among age group 6 – 10 years represented 159/231 (68.83%), followed by >10 years 46/231 (19.91%) and ≤ 5 years 26/231 (11.26%) as in **Fig 3.3.4.** For 6 – 10 years age group, the major infections were found by *Strongylus*, Mixed infection, *Dictyocaulus* and *Cyathostomes*. And for the age group >10 years, the main parasites were *Strongylus*, followed by Mixed infection and *Strongyloides*. Whereas, for ≤ 5 years age group, the most important helminthes were *Strongylus*, *Dictyocaulus and* mixed infection as in **Table 3.3.3**. Moreover, according to the ratio of male to female in working donkeys screened, there was no statistically significant difference in infection according to sex.

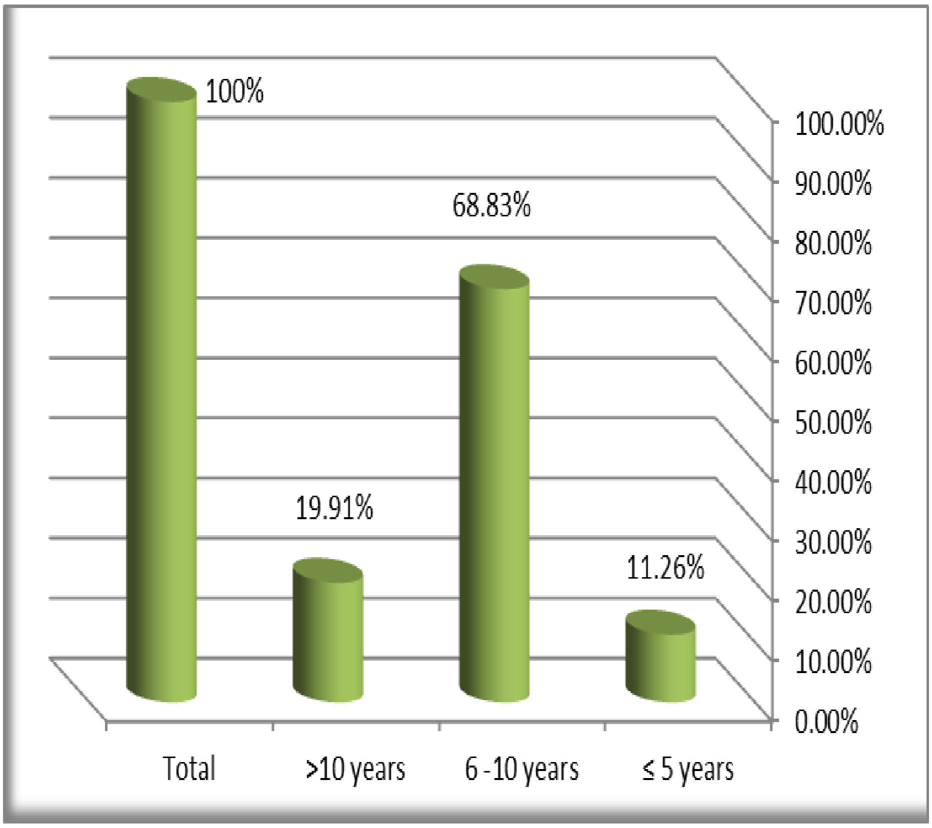


Figure 3.3.4: Helminthic burden on draught donkey's age groups in the study area

Table 3.3.3: Distribution of helminth according to animals' age groups

parasite	Age group ≤ 5 years		6 -10 years		>10 years		Total
	Frequency	%	Frequency	%	Frequency	%	
Strongylus	13	50.00	76	47.80	26	56.52	115
Cyathostomes	1	03.85	10	06.29	2	04.35	13
Oxyuris	1	03.85	5	03.14	2	04.35	8
Anoplocephla	1	03.85	4	02.51	0	00.00	5
Parascaris	1	03.85	4	02.51	1	02.17	6
Dictyocaulus	5	19.23	15	09.43	0	00.00	20
Strongyloides	1	03.85	6	03.77	5	10.90	12
Mixed infection	3	11.53	38	23.90	9	19.57	50
Trichostrongylus	0	00.00	1	00.63	1	02.17	2
Total	26		159		46		231
	(11.26%)	100.00	(68.83%)	100.00	(19.91%)	100.00	(100%)

3.3.2. Intensity of helminth infection

As severity of helminth infection is concerned, the overall mean egg per gram count (epgc) was 704.14±116.59 with a range of 50 - 10200 (epg). The highest egg per gram count was found in dry hot season. The infection was found 316/459 (68.85%) for mild, 82/459 (17.86%) for severe and 61/459 (13.29%) for moderate infections as in **Fig 3.3.5**. The great majority of mild infection was found in *Strongylus spp* 199/316 (62.97%), followed by mixed infection 31/316 (9.81%), *Dictyocaulus* 27/316 (8.54%) and *Strongyloides* 18/316 (5.70%). For severe helminth infection, the higher percentage was found also in *Strongylus*, then mixed infection, *Cyathostomes* and *Strongyloides*, with percentages 41/82 (50%), 28/82 (34.14%), 4/82 (4.88%) and 4/82 (4.88%), in the same order. In addition, the major moderate helminth infections were found as follows: 35/61 (57.38%), 18/61 (29.50%) and 3/61 (4.92%) for *Strongylus*, mixed infection and *Cyathostomes*. correspondingly. Moreover, it was found that, *Anoplocephla*, *Paranoplocephla and Trichostrongylus* were found in the mild infection only *as* in **Table 3.3.4**.

The mean epg (704.14±116.59) in the study area was approximately around that of mild and moderate infection (800-1000) epg according to Soulsby (1982). The great majority of eggs were that of *Strongylus spp*, followed by *Dictyocaulus spp, Strongyloids spp, Cyathostomes, Oxyuris spp, Parascaris spp, Anoplocephla spp, Paranoplocephla, spp* and *Trichostrongylus spp* **(plate 3.3.2 and 3.3.3)**.

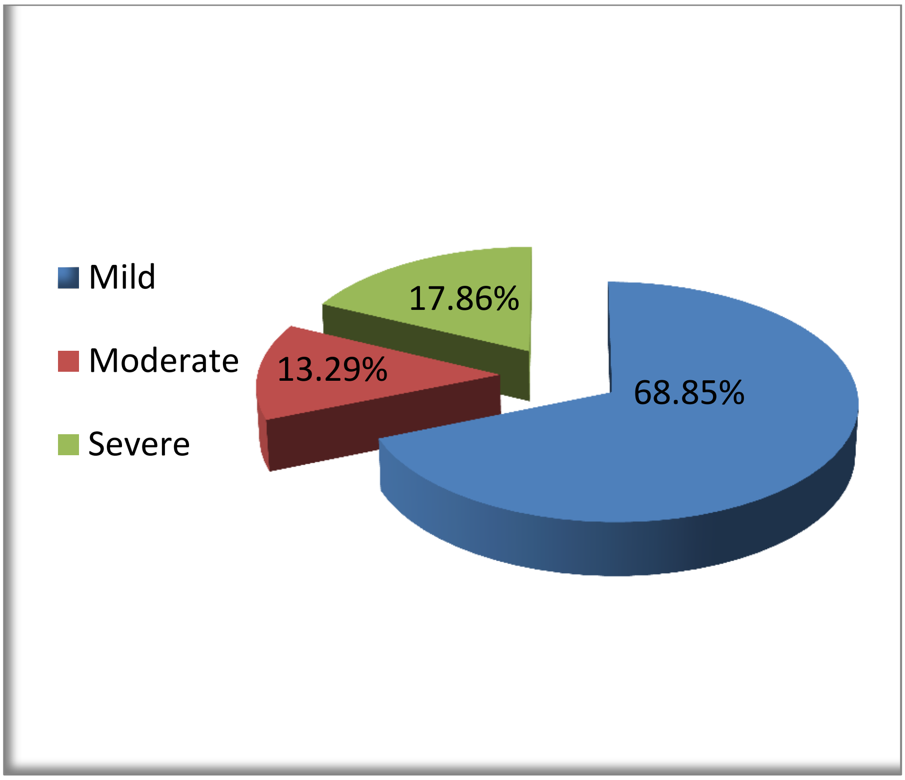


Figure 3.3.5: Severity of helminth infections in the study area

Table 3.3.4: Distribution of helminth by severity of infection

	Helminth infection						
Parasite	Mild		Moderate		Severe		Total
	Frequency	%	Frequency	%	Frequency	%	
Strongylus	199	62.97	35	57.38	41	50.00	275
Cyathostomes	9	02.85	3	04.92	4	04.88	16
Oxyuris	13	04.11	2	03.28	1	01.22	16
Anoplocephla	5	01.58	0	00.00	0	00.00	5
Parascaris	9	02.85	2	03.28	1	01.22	12
Dictyocaulus	27	08.54	0	00.00	3	03.66	30
Strongyloides	18	05.70	1	01.64	4	04.88	23
Paranoplocephla	3	00.95	0	00.00	0	00.00	3
Mixed infection	31	09.81	18	29.50	28	34.14	77
Trichostrongylus	2	00.63	0	00.00	0	00.00	2
Total	316		61		82		459
	(68.85%)	100.00	(13.29%)	100.00	(17.86%)	100.00	(100%)

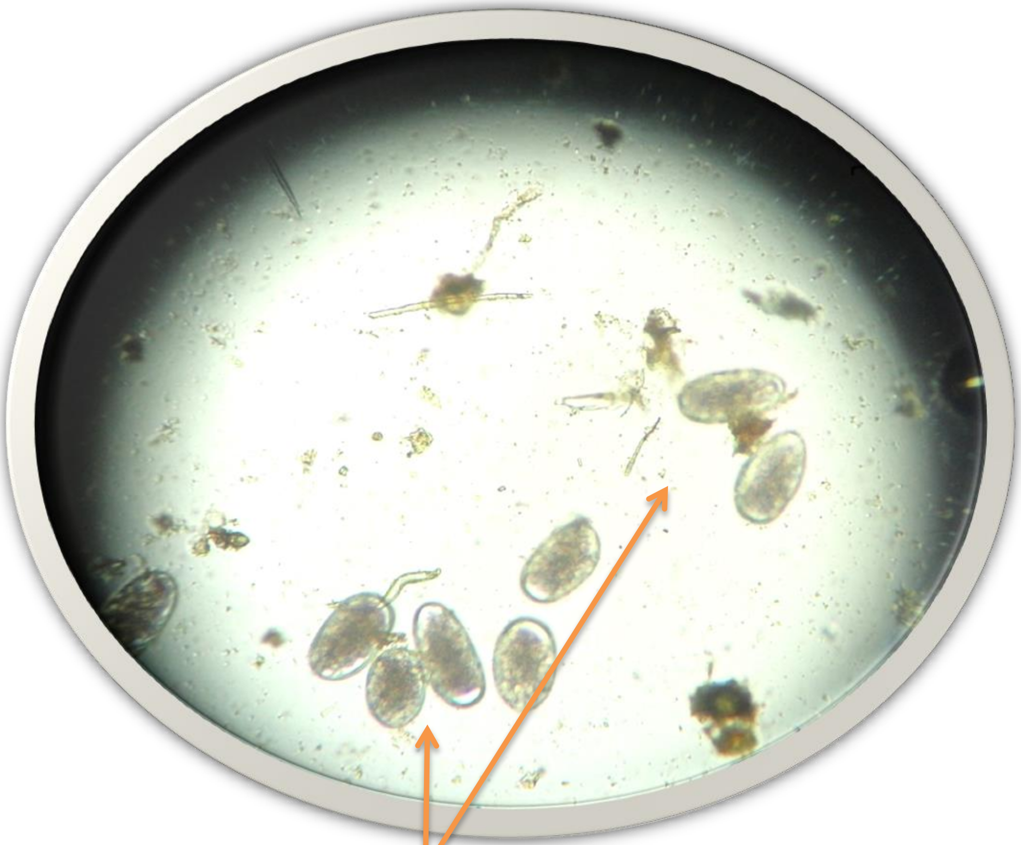
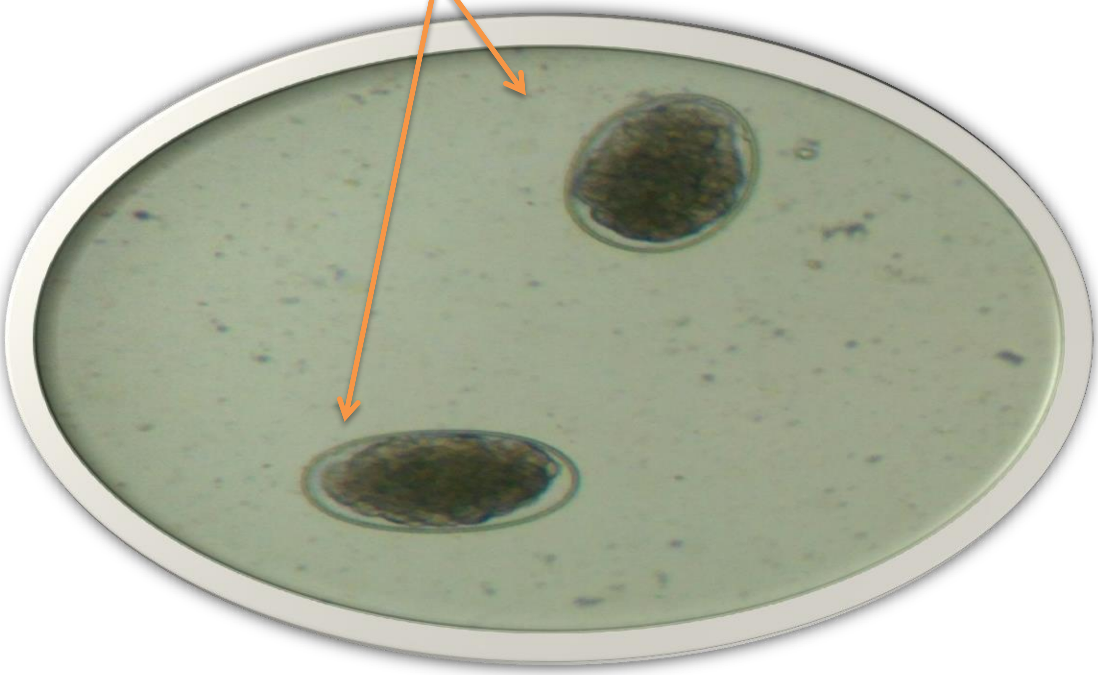


Plate 3.3.2: *Strongylus spp* egg in infected donkeys



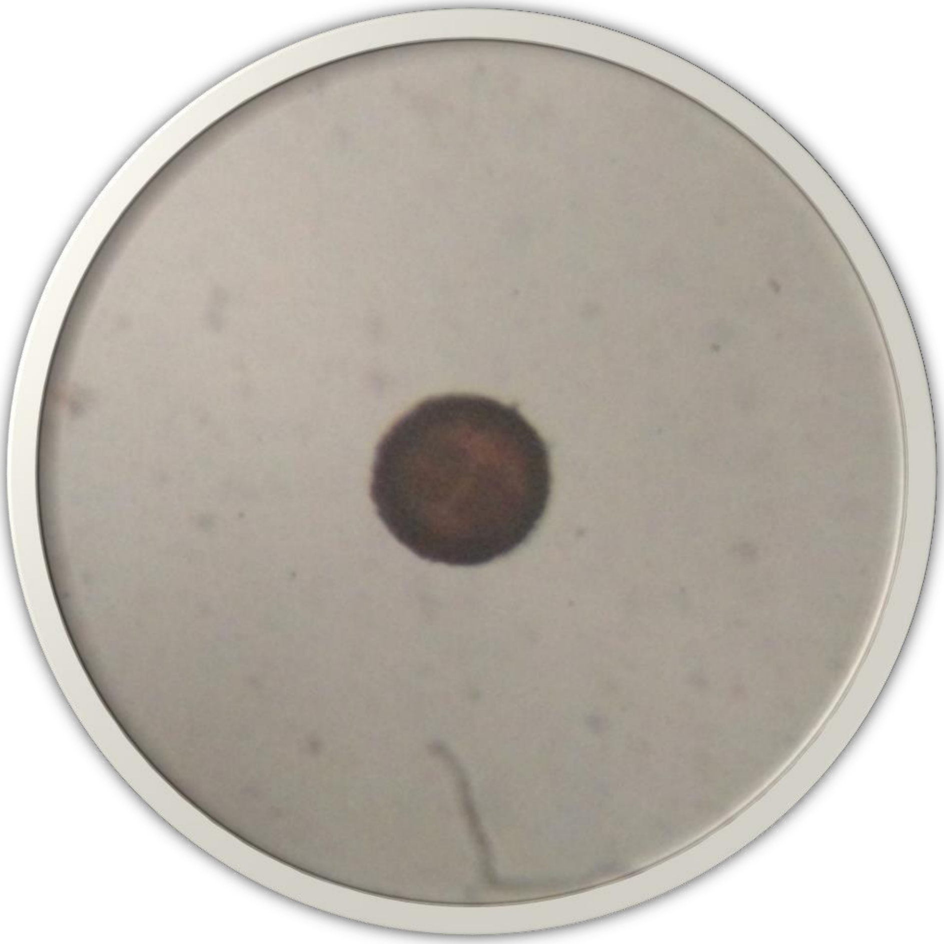


Plate 3.3.3: ***Parascaris spp*** **egg found in infected donkey**

3.3.3. Packed cell volume (PCV) determination

The value of mean packed cell volume in study area was found to be 32.38±7.37. The minimum and maximum values of PCV in the study area were 17 and 50 respectively. For the negative examined animals, the mean PCV was 32.10 and for the positive results, the mean PCV was 32.82. Therefore, PCV difference was not statistically significant. Also, the value of PCV was not statistically different between parameters of sex, age, and body condition.

3.4. Discussion

The objective of the study was to study the prevalence rates of helminth infestation, identify the pathogenic helminth parasites and to compare the findings between Ghebaish and Alfula localities in draught donkeys in in West Kordofan.

In this study, the overall prevalence rate was found representing (38.25%) and prevalence rates in Ghebaish and Alfula were (20.25%) and (18%), respectively. When each locality was taken individually, the prevalence rates were; 243/600 (40.50%) for Ghebaish and 216/600 (36%) for Alfula. In Sudan, these results tend to be lower than the results found by Najat (2006) and Abdurhman *et al*. (2016). This may be due to the large number of animals examined in this study compared to the small number of animals examined in Khartoum State (100 animals) and Nyala (92 animals). The findings were also, lower than the results found by Seri *et al*. (2004); however the two studies were examined the same number (1200) working donkeys. This may be attributed to existence and massive usages of anthelmintics nowadays and the study was conducted on all working donkeys with both sexes beside the difference in environment and management systems between West Kordofan and Khartoum.

In another way, the overall prevalence of this study was found approximately equal or near to the results found by Sawsan *et al*. (2006) and Adam *et al*. (2013). This may be referred to similarity in environment and human activities between West Kordofan and South and North Darfur States.

The overall prevalence of this study was found lower than many studies conducted in Africa, for example; Mezgebu *et al*. (2013); Tsegaye and Chala (2015); Wako *et al*. (2016); Belay *et al*. (2016); Mohammed *et al*. (2016); Gebreyohans *et al*. (2017); Kassa and Zeleke (2017). This may be due to vast difference in environment and culture of donkey users between Sudan and the rest of African countries.

Nine Helminth parasites were identified in this study included: *Strongylus spp, Dictyocaulus spp, Strongyloids spp, Cyathostomes, Oxyuris spp, Parascaris spp, Anoplocephla spp, Paranoplocephla, spp* and *Trichostrongylus spp*. With dominance (high prevalence rate) of *Strongylus spp* followed by mixed infection and *Dictyocaulus spp*.

These findings and the dominance of *Strongylus spp* agree with a lot of studies conducted in Sudan such as Sawsan *et al*. (2006); Adam *et al*. (2013); Molla *et al*. (2015); Abdurhman *et al*. (2016). *Strongyles* were most prevalent and pathogenic nematodes that cause one of the most important internal parasitic diseases of equines (López-Olvera *et al*., 2006; Canberra Equine hospital, 2013; Tesfu *et al*., 2014; Khan *et al*., 2015; Waqas *et al*., 2015). The clinical signs of strongylosis are often nonspecific and usually depend on the age of the animal and the severity of the infection (Clark, 2008). Adult worms of large Strongyles as well as the migrating larvae are the most harmful worm species to donkeys particularly *Strongylus vulgaris*, whose larvae causes extensive damage to the mesenteric artery and its branches. Large Strongyles can also cause damage to the gut; hence can lead to diarrhea and subsequent dehydration, colic, anaemia, loss of appetite (anorexia), depression, and weight loss. In severe cases gangrenous enteritis, intestinal stasis (i.e. arrest or delay of the intestinal food passage), rupture and intestinal infarct may happen, that may end with a fatal outcome. Therefore, the effects of strongylosis can be devastating. This statement consolidate the findings of Abdel Wahab *et al.* (2014) in three outbreaks of a death causing disease in donkeys in South and West Darfur States, where they found all faecal samples revealed heavy infection of *strongyles* (>3000 eggs per gram). Furthermore, post mortem examination showed acute gastroenteritis and inflammation of the mesenteries, and *Strongylus vulgaris*, *S. edentatus* and *S. equinus* were found in the stomach.

Regarding to the seasonal distribution of infections, most of them were found during dry hot season followed by wet hot and dry cool. *Strongylus spp* was found predominant in the three seasons, high in the dry hot followed by dry cool and then wet hot season. Cyathostomes were appeared only during wet season, whereas *Trichostrongylus* was found in the dry hot season only.

These results vary from the results found by Seri *et al*. (2004); gastrointestinal parasites were more prevalent in cold season (72.5%) than hot (69.3%), and rainy season (68.5%). This can be explained by the difference in the management practices between Kordofan and Khartoum and absence of good vegetation in Kordofan during dry hot season, which predispose gastrointestinal parasites infection. Which agree with (Pearson and Mohammed, 2000) where they stated; emaciation is the biggest problem facing donkeys kept in the hotter tropical areas where food is in short supply and of poor quality for many months of the year. These donkeys often work for at least part of each day in transport or tillage activities. Donkeys working in urban areas, where there is less opportunity for grazing can be more at risk of malnutrition than those working in rural areas. Also, the results of this study differ from the results obtained by Abdurhman *et al*. (2016), where there were no significant differences was observed between different seasons. This is may be due to the few number of donkeys examined (92 donkeys) and all of them from Nyala livestock market.

The study was also revealed that, The majority of infected animals were found among age group 6 – 10 years representing (68.83%), followed by >10 years (19.91%) and ≤ 5 years (11.26%) age groups. This can be explained as; the 6 – 10 years age group was the most commonly utilized in intensive work, which supposed to be, predisposing factor for the infection. These findings consolidate with the results of risk factors in this study (chapter 4), where the work was more intensive in 6-10 years age group with percentage of (52%) followed by <5 years (38%) then >10 years (10%). These results not agree with Mohammed *et al*. (2016) where they found that; season, age and sex were and were not statistically significant. This may be returned to the difference in environment and management practices between Sudan and Nigeria.

3.5. Conclusion

The study revealed that donkeys in the study area were infected with a variety of helminth, which were representatives of the important equine pathogenic parasites found in Sudan. Nine Helminth parasites were identified in this study included: *Strongylus spp, Dictyocaulus spp, Strongyloids spp, Cyathostomes, Oxyuris spp, Parascaris spp, Anoplocephla spp, Paranoplocephla, spp* and *Trichostrongylus spp.* With dominance (high prevalence rate) of *Strongylus spp* followed by mixed infection and *Dictyocaulus spp*.

The prevalence of *Strongylus spp* and mixed infection were found to be significantly higher in the two regions mainly in Ghebaish during the dry hot season.

Chapter four

Risk factors assessment for susceptibility to helminth infections

4.1. Introduction

Sudan's equines are important in pastoralism and agriculture as riding, work and transport animals and in urban areas for transport. In 2010 Sudan had 7.5 million donkeys and 0.8 million horses. Limited disease diagnosis and treatment was then assured by the civilian Sudan Veterinary Service and a research arm was established in the 1920s. Disease diagnosis, treatment and control are now secured by public and private services acting within laws governing diseases and welfare (Trevor, 2017).

There is complete lack of veterinary service provision to donkeys and lack of awareness of animal welfare (Nakayima *et al*., 2017) Therefore, Public awareness creation to equine owners on proper deworming, sufficient feed supply and minimizing extensive open grazing of donkeys and horses is important. Also balancing of the work load and duration should be managed (Tesfu *et al.*, 2014). Donkeys continue to contribute to national and household economies, but do not receive services equivalent to their inputs. Therefore, the objectives of this study were:

1- To identify the most important risk factors associated with helminth infections in draught donkeys in West Kordofan.

2- To deduce measures that can contribute in future awareness programs for control of gastrointestinal parasites in draught donkeys in West Kordofan.

4.2. Materials and methods

4.2.1. Donkey owners' information

A questionnaire had been developed, tested and applied. The questionnaire aimed at collecting data from donkey owners to cover the following areas; personal data and risk factors. The risk factors covered were; animal factors, Management factors, Environmental factors and utilization.

The animal factors were; age, sex, type, body condition and faecal consistency. Management factors include; feeding type, method and habits, housing, bedding and disposal of manure. Knowledge about presence of helminth, use of anti-helmintics, treatment response, source of treatment and presence of other infections were also covered. Environmental factors include the status of vegetation throughout the different seasons. Utilization comprised; work intensity, type of work, donkey's user and usage. Other commends were also enclosed (**Appendix 2)**.

Total of (600) draught donkey owners were responded to the questionnaire. The questionnaires data were entered a computer and analysed.

4.3. Data analysis

Data were collected and arranged in standard master sheet then entered a computer software statistical package for social studies (SPSS) (SPSS version 16). Frequencies and Chi Squire Test were obtained. P value less than 0.05 was considered as statistically significant.

4.4. Results

A total of 600 questionnaires were analyzed, and the analysis revealed that, (231) donkeys out of the (600) animals which owners responded to the questionnaires were found positive with prevalence rate of 231/600 (38.50%). The majority of the donkeys were found in good and moderate body condition. Also it revealed that the majority of positive screened donkeys were in good body condition followed by moderate and then bad body condition, because the great majority of helminth infections were mild. When positive results compared with negative results, it was found that, the positive results were higher, mainly in moderate and bad body condition as in **Fig 4.4.6**.

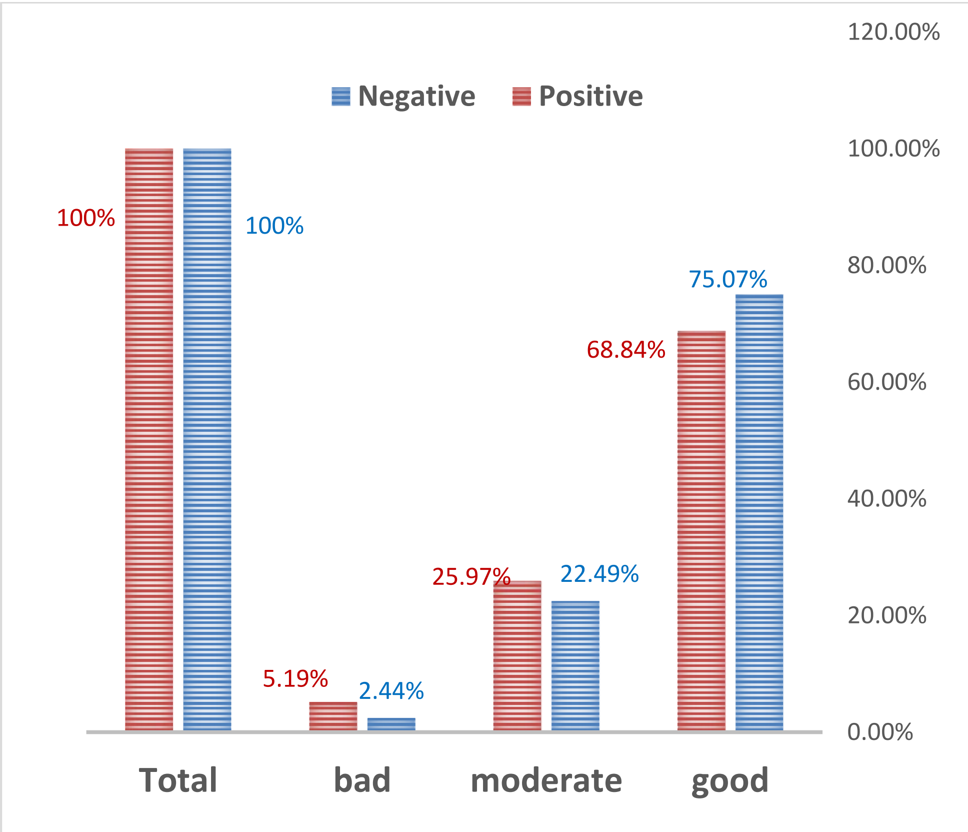


Figure 4.4.6: Description of the body condition by infected and non-infected draught donkeys

The great majority of donkey owners who responded to the questionnaire mentioned that; female working donkeys usually were used for riding in rural areas for individual transport, communication and for low work intensity like carrying water, marketing, etc.

Table 4.4.5 illustrating distribution of the management factors by helminth infections as mentioned by the working donkey owners. A total of 449/600 (74.83%) of respondents who participated in the questionnaire said they feed their animals indoor, of which 195/231 (84.42%) donkeys were found positive, while the rest 151/600 (25.17%) of the respondents were feeding their donkeys outdoor, of which 36/600% (15.58%) were found positive as illustrated in **Fig 4.4.7**. Regarding the feeding type, most of helminth infections appeared in donkeys that were fed on grass (52.38%) followed by those fed with both (45.89%) and supplementation (01.73%). The same way for negative results. For bedding usage, helminth infection was found higher in the sandy bedding which was 185/231 (80.09%) followed by no bedding (free animal) 35/231 (15.15%) then hay or straw 11/231 (4.76%) as in **Fig 4.4.8.** Regarding housing type, most of the positive results were found in the tethered donkeys 142/231 (61.47%), then zariba 55/231 (23.81%) and free 34/231 (14.72%).

Table 4.4.5: Distribution of the management factors by helminth infections

Variable	Negative		Positive		Total	
	Frequency	%	Frequency	%	Frequency	%
Feeding habits						
Indoor	254	68.83	195	84.42	449	74.83
Outdoor	115	31.17	36	15.58	151	25.17
Total	369	100.00	231	100.00	600	100.00
Feeding type						
grass	233	63.14	121	52.38	354	59.00
Supplementation	09	02.44	04	01.73	13	02.17
Both	127	34.42	106	45.89	233	38.83
Total	369	100.00	231	100.00	600	100.00
Bedding						
Sand	259	70.19	185	80.09	444	74.00
Hay or Straw	30	08.13	11	04.76	41	06.83
Not available	80	21.68	35	15.15	115	19.17
Total	369	100.00	231	100.00	600	100.00
Housing type						
Zariba	121	32.79	55	23.81	176	29.33
Tethered	161	43.63	142	61.47	303	50.50
Free	87	23.58	34	14.72	121	20.17
Total	369	100.00	231	100.00	600	100.00

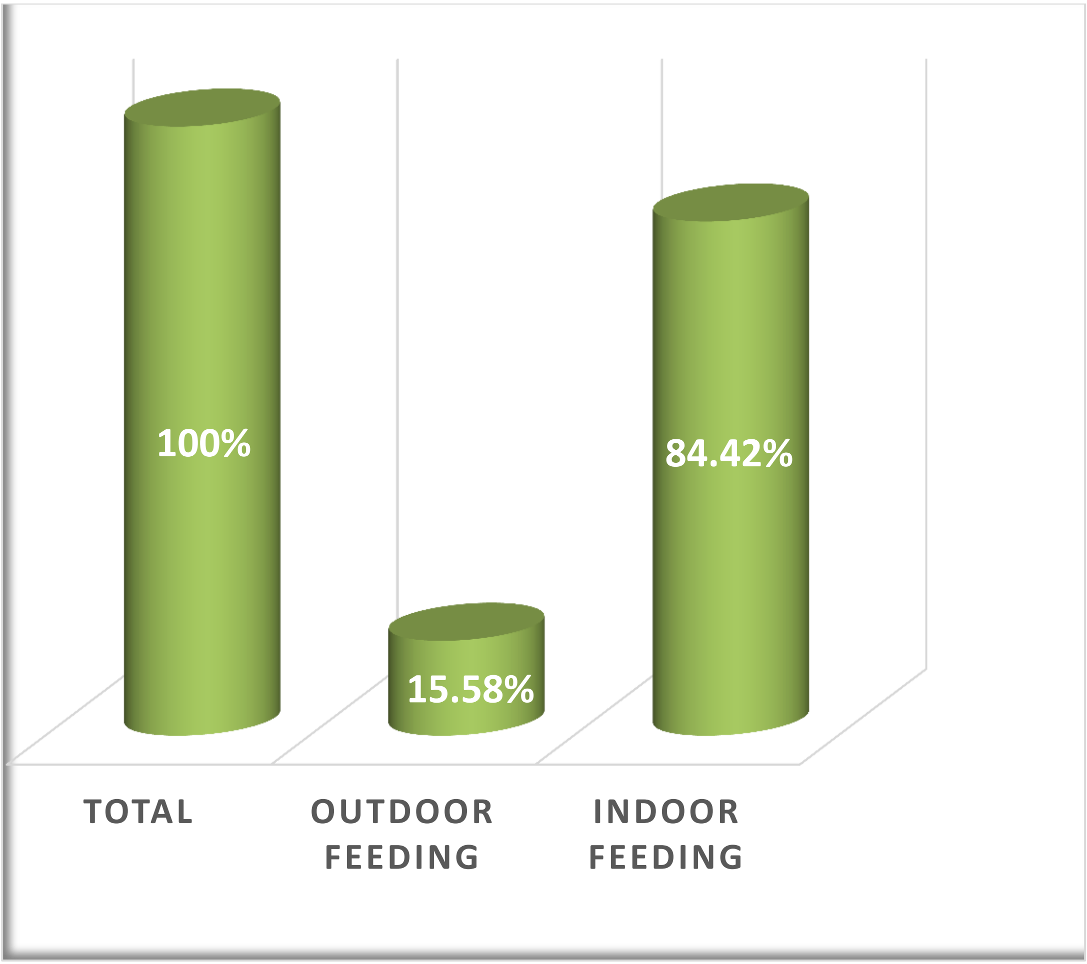


Figure 4.4.7: Description of the infected draught donkeys by method of feeding

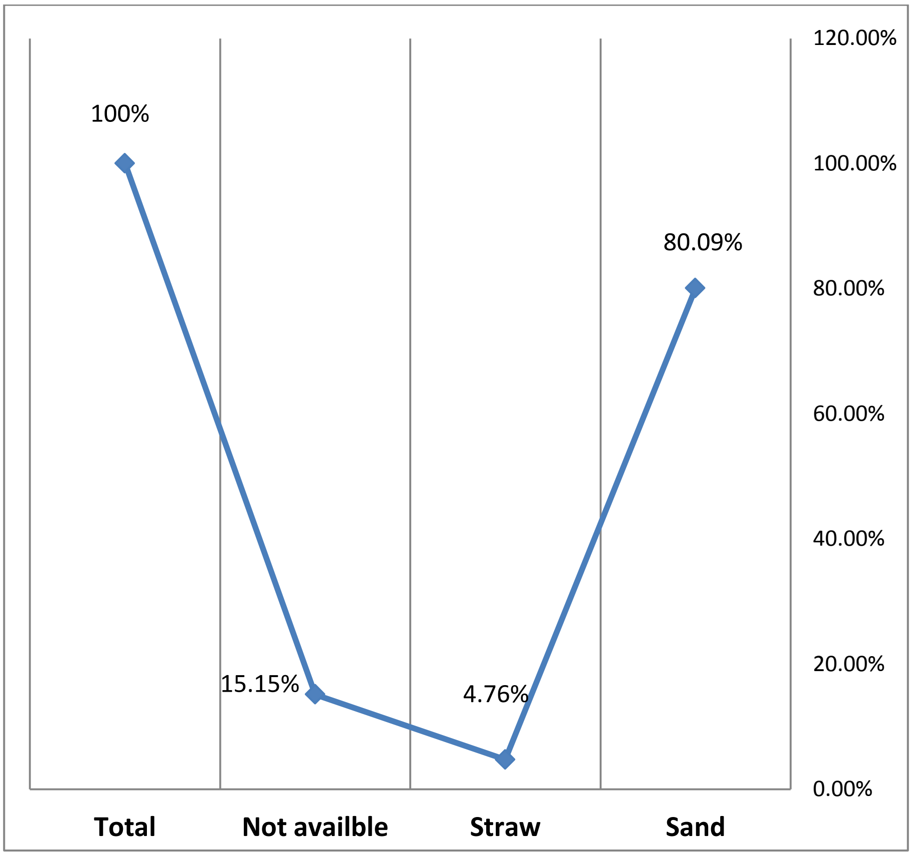


Figure 4.4.8: Description of helminth infections by bedding type

Regarding disposal of manure 432 out of 600 respondents said (yes) with percentage of 432/600 (72%) and the rest 168/600 (28%) said (no). The majority of infected animals their owners found used to clean their animal bedding from manure with percentage of 188/231 (81.39%), and 43/231 (18.61%) were not get rid of manure, whereas 244/369 (66.12%) and 12/369 (33.88%) for who said (yes) and (no), respectively were found without infections.

For frequency of disposal of manure, the great majority of the respondents used to clean their animal bedding weekly with percentage of 322/600 (53.67%) followed by no frequency, monthly and cleaning at specific time with percentages of 168/600 (28%), 57/600 (9.5%) and 53/600 (8.83%), respectively. Regarding infections, the highest infection was found in weekly cleaning with percentage of 140/231 (60.61%) followed by no frequency, monthly and cleaning at specific time with percentages of 43/231 (18.61%), 30/231 (12.99%) and 18/231 (07.79%), respectively. However, 182/369 (49.32%), 125/369 (33.88%), 35/369 (09.48%) and 27/369 (07.32%) weekly cleaning, no frequency, specific time and monthly in this manner were found negative as in **Table 4.4.6**.

Table 4.4.6: Distribution of disposal of manure by helminth infections

Variable	Negative		Positive		Total	
	Frequency	%	Frequency	%	Frequency	%
Disposal of manure						
Yes	244	66.12	188	81.39	432	72.00
No	125	33.88	43	18.61	168	28.00
Total	369	100.00	231	100.00	600	100.00
Frequency of Disposable of manure						
No frequency	125	33.88	43	18.61	168	28.00
Monthly	27	07.32	30	12.99	57	09.50
Weekly	182	49.32	140	60.61	322	53.67
Specific time	35	09.48	18	07.79	53	08.83
Total	369	100.00	231	100.00	600	100.00

Table 4.4.7 describing distribution of helminth infection by owners' knowledge about overall infection management. When the questionnaire respondents asked about their knowledge about helminthic infection and usage of anthelmintic drugs, 146 out of 231 donkey owners of the positive cases mentioned that, they don't know helminth infection 146/231 (63.20%), and the rest (85) owners have an idea about helminth infections 85/231 (36.80%), therefore, the helminth infections (positive results) were found lower in donkeys of those who said; yes, for treatment, and higher in animals that their owners said; no or not use anthelmintics.

Regarding use of anthelmintic treatments, 365 out of 600 respondents answered no, when asked about the use of anthelmintics with percentage of 365/600 (60.83%) and the rest answered yes with percentage of 235/600 (39.17%). When focusing on the positive results, the higher percentage was found in the animals of those answered no (i.e. not using treatment) with percentage of 136/231 (58.87%) and the rest 95/231 (41.13%) for animals of the respondents who answered yes.

For treatment response, (360) respondents out of 600 had no idea (no answer) 360/600 (60%), 205/600 (34.17%) mentioned that, their animals were cured and 35/600 (05.83%) answered no change after treatment. The high positive results were found at those had no idea,
with percentage of 136/231 (58.87%) followed by who answered (cured) 85/231 (36.80%) and finally who answered no change 10/231 (04.33%).

Also for the source of treatment 362 out of 600 respondents answered, they had no idea (no answer) about the sources of treatment with percentage of 362/600 (60.33%) and the rest took the anthelmintic treatments from pharmacies 195/600 (32.50%), followed by friends 22/600 (03.67%) and clinics 21/600 (03.50%). The great majority of the positive samples were found in animals of owners who were with no idea with percentage of

136/231 (58.87%) followed by pharmacy 78/231 (33.77%), clinic 10/231 (04.33%) and friend 7/231 (03.03%).

Table 4.4.7: Distribution of helminth infection by owners' Knowledge about overall infection management

Variable	Negative		Positive		Total	
	Frequency	%	Frequency	%	Frequency	%
Knowledge about presence of helminth infection						
Yes	128	34.69	85	36.80	213	35.50
No	241	65.31	146	63.20	387	64.50
Total	369	100.00	231	100.00	600	100.00
Use of anthelmintic treatments						
Yes	140	37.94	95	41.13	235	39.17
No	229	62.06	136	58.87	365	60.83
Total	369	100.00	231	100.00	600	100.00
Treatment response						
No answer	224	60.70	136	58.87	360	60.00
Cured	120	32.52	85	36.80	205	34.17
No change	25	06.78	10	04.33	35	05.83
Total	369	100.00	231	100.00	600	100.00
Source of treatment						
No answer	226	61.25	136	58.87	362	60.33
Vet. Pharmacy	117	31.71	78	33.77	195	32.50
Clinic	11	02.98	10	04.33	21	03.50
Friend	15	04.06	7	03.03	22	03.67
Total	369	100.00	231	100.00	600	100.00
Presence of other infections						
No	292	79.13	193	83.55	485	80.83
Yes	77	20.87	38	16.45	115	19.17
Total	369	100.00	231	100.00	600	100.00

Regarding presence of other infections, the majority of the respondents answered; no for both negative and positive animals, hence, presence of other infection had no effect on helminth infections among working donkeys in the study area.

Concerning environmental factors; the positive results of faecal samples examination were found lower in good vegetation (during wet and dry cool) season than negative results, whereas in poor vegetation, the positive were found higher than the negative results as illustrated in **Fig 4.4.9**.

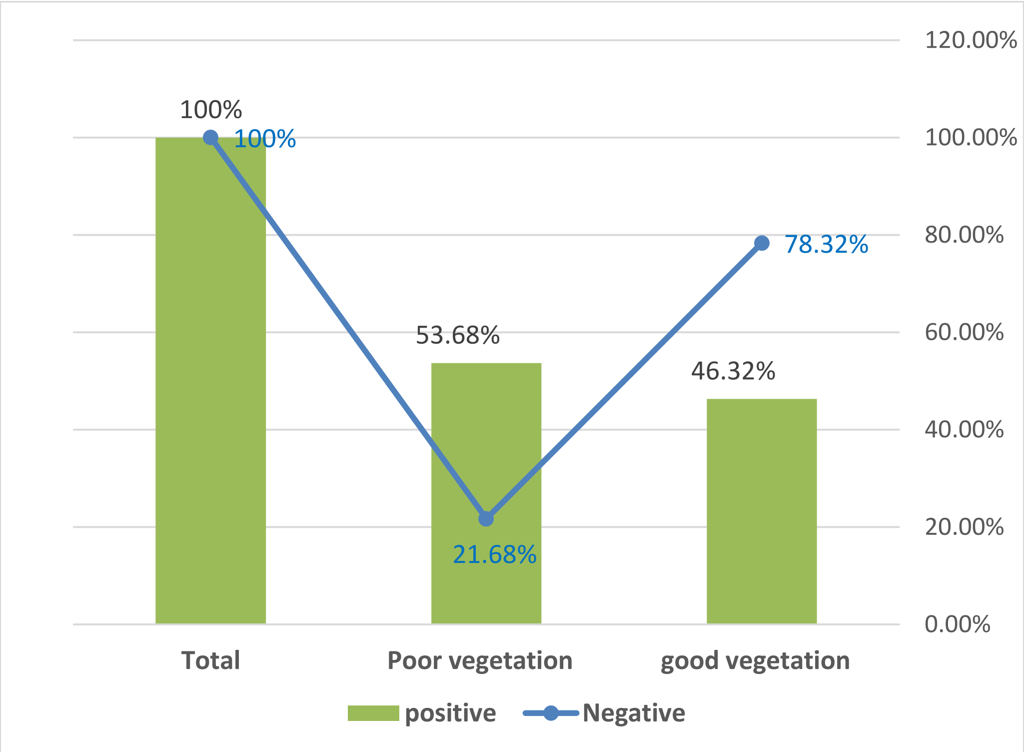


Figure 4.4.9: Description of infection status by vegetation status

Table 4.4.8 illustrating distribution of utilization of draught donkeys by helminth infections. Draught donkeys were found mostly used by adult men with percentages of 332/600 (55.33%) followed by children 157/600 (26.17%), more than one user 82/600 (13.67%) and women 29/600 (04.83%). And in the same manner, the results of sampled animals were found high in animals used by adult men, then children, more than one user and women with percentages of (41.99%, 63.68%), (37.66%, 18.97%), and (15.59%, 12.47%) and (04.76%, 04.88%) for positive and negative results, respectively.

For the work type, the animals were used mostly for draught, followed by multiple works and riding with percentages 287/600 (47.83%), 172/600 (28.67%) and 141/600 (23.50%) correspondingly. Also, the results of samples examined were found higher in animals used for draught followed by multiple works and riding with percentages of (52.81%,44.72 %), (23.81%, 31.70%) and (23.38%, 23.58%) for positive and negative results, correspondingly.

The intensity of work as general was found mostly low 299/600 (49.83%) followed by moderate 251/600 (41.83%) and intensive 50/600 (8.34%). The results of samples surveyed were found higher in animals with low work followed by moderate and intensive work with percentages of (46.32%,52.03 %), (48.48%, 37.67%) and (05.20%, 10.30%) for positive and negative results, in this way.

Moreover, the majority of draught donkeys were found used in the rural areas; with percentages of 312/600 (52%) for rural and 288/600 (48%) for urban. The positive results was found higher in donkeys used at urban areas than those used at rural areas with percentages of 124/231 (53.68%) and 107/231 (46.32%) for urban and rural areas, respectively.

Table 4.4.8: Distribution of utilization of draught donkeys by helminth infections

Variable	Negative		Positive		Total	
	Frequency	%	Frequency	%	Frequency	%
User						
Adult man	235	63.68	97	41.99	332	55.33
Child	70	18.97	87	37.66	157	26.17
Woman	18	04.88	11	04.76	29	04.83
More than one	46	12.47	36	15.59	82	13.67
Total	369	100.00	231	100.00	600	100.00
Work type						
Draught	165	44.72	122	52.81	287	47.83
Ridding	87	23.58	54	23.38	141	23.50
Multiple	117	31.70	55	23.81	172	28.67
Total	369	100.00	231	100.00	600	100.00
Work intensity						
Low	192	52.03	107	46.32	299	49.83
Moderate	139	37.67	112	48.48	251	41.83
Intensive	38	10.30	12	05.20	50	08.34
Total	369	100.00	231	100.00	600	100.00
Usage site						
Urban	164	44.44	124	53.68	288	48.00
Rural	205	55.56	107	46.32	312	52.00
Total	369	100.00	231	100.00	600	100.00

Regarding the age groups, it was found that; 6-10 years age group was the most used group with percentage of 377/600 (62.83%), followed by >10 years 113/600 (18.83%) and <5 years 110/600 (18.34%). Furthermore, when the intensive work was concerned, it was found that; the work was more intensive in 6-10 years age group with percentage of 26/50 (52%) followed by <5 years 19/50 (38%) then >10 years 5/50 (10%) as in **Table 4.4.9**.

Table 4.4.9: Distribution donkeys' age by work intensity

	Work intensity							
Age group	Low		Moderate		Intensive		**Total**	
	frequency	%	frequency	%	frequency	%	frequency	%
<5 years	39	13.04	52	20.72	19	38.00	110	18.34
6-10 years	198	66.22	153	60.96	26	52.00	377	62.83
>10 years	62	20.74	46	18.32	05	10.00	113	18.83
Total	299 (49.83%)	100.00	251 (41.83%)	100.00	50 (8.34%)	100.00	600 (100%)	100.00

4.5. Discussion

The objective of the present study was to identify the most common risk factors associated with helminth infections in draught donkeys, and to provide findings that can contribute in future awareness programs for control of gastrointestinal parasites in West Kordofan.

As known donkeys play a vital role on Sudanese livelihood, especially to the rural communities. Working donkeys are multipurpose animals; they provide unlimited services including transportation, drafting of carts, packing, riding, agricultural cultivation and livestock guarding. Furthermore, donkeys were used in social activities such as communication between villages, towns, etc. Some of people in the rural communities feel proud when one owned an expensive and faster donkey. Furthermore, donkeys are docile, quiet and very hardy animals, can work continually on adverse conditions with little rest and on poor management system. All the above mentioned roles are in agreement with many researchers and organizations: Kataria and Kataria (2010); FAO (2011); Yilmaz (2012); Madure (2014); Valette (2015); Knottenbelt and Deceim (2015); The Donkey Sanctuary (2017). Moreover, in some European countries and China, donkey products; meat, milk and skins were used in variety of purposes as mentioned by: Karatosidi *et al*. (2013); Zhu Wenqian (2015); Ragona *et al.* (2016); China daily (2016). Accordingly, I can say; donkeys are indispensable and very significant animal for human being all over the world.

Nevertheless, donkeys are still neglected animals, especially health and management practices. In Sudan as general and in West Kordofan in particular, working donkeys are facing many problems related to the owners; such as insufficient knowledge of proper management, misusage and poor health care. Thus, a questionnaire was designed in order to evaluate the risk factors related to draught donkeys in west Kordofan State. Great majority of the respondents mentioned that; the female working donkeys usually were used for riding in rural areas for individual transport and communication and for low work intensity like carrying few litres of water and personal marketing purposes. This was appositive point as managemental practice in the study area.

The study revealed that the majority of positive screened donkeys were found in good body condition followed by moderate and then bad body condition, because the great majority of helminth infections were mild. When positive results compared with negative results, it was found that the positive results were higher, mainly in moderate and bad body condition animals. This can be explained that, the poor body condition is an indication for infection and the poorness of body condition predispose the parasitic infection, which agree with: Pearson and Mohammed (2000); Rickard (2001); Yoseph *et al.* (2005); Khan *et al.* (2015); Rasool *et al*. (2016). In this study it was found that, the great majority of animals were fed indoor, and were found with highly infected, whereas those were fed outdoor, were found with low infection rate. This may be attributed to presence of the source of infection at indoor places and there was high chance to get the infection. These findings agree with Polley (2010).

Helminth infections were found higher in the sandy bedding which was (80.09%) followed by no bedding (free animal) (15.15%) then hay or straw (4.76%). Also, for housing type high infection rate was found in tethered donkeys with percentage of (61.47%), followed by kept in zariba (fence) (23.81%) and left free (14.72%). These results may be attributed to sandy environment which was appropriate for the parasites, and were found in the same way as Polley (2010) who mentioned; hosts, parasites and the local environment are intimately linked. Also, these results were agree with Magzoub (2002) for the fact that; the structure and type of the grass depend upon soil type, and that determine the degree of formation of the (mat) between the soil and the grass. The mat is mainly found at the old grazing lands, and it keeps the high humidity for weeks in dry seasons, which decreases the change of temperature, and helps in presence and growth of larvae of parasites.

In this study although the majority of donkey owners were found disposing manure weekly, but high helminth infections were found in their animals. This may be attributed to the level of disposal and the sandy soil which difficult to clean and mat may be formed which keeps humidity.

As the knowledge of draught donkey owners about helminth infection and use of treatment is concerned, the great majority of working donkey owners was found ignoring the presence of helminth infections and the use of anthelmintic treatments, and had no clear idea about the response of the treatment. This means; there was a problem in drug administration and had no enough knowledge about treatment of parasitic infections. Therefore, there was high need for extension work among working donkeys users communities. These findings agree with: Wubie and Getaneh (2015); Belay *et al*. (2016); Rasool *et al.* (2016); Mohammed *et al*. (2016); Takele and Sisay (2016); Gebreyohans *et al*. (2017); Kassa and Zeleke (2017).

Regarding the sources of treatments, in this study, the great majority of the positive samples were found in animals of owners who were with no idea with percentage of (58.87%) followed by pharmacy (33.77%), clinic (04.33%) and friend (03.03%). According to these findings it is of great importance that all sources treatments must be managed by well-trained veterinarians in order to direct the appropriate drug and write dose. Furthermore, veterinary clinics should be improved and strengthened with equipments and skilled labors. Therefore, the animal owners can get back the confidence and satisfaction with the veterinary medical institutions.

Concerning environmental factors, the results of the respondents to the questionnaire revealed that; the highest percentage of infection was in poor vegetation. These results match the results of the seasonal distribution of infections, where most of the infections were found during dry hot season where there was poor vegetation which act as risk factor that predispose parasitic infection. These results were agree with Guyo *et al*. (2015), and in contrast were not agree with Abdurhman *et al*. (2016) where they found that; the hot wet season is most preferable for development and survival of the recovered parasites. This can be correlated to great difference in number of animal examined between the two studies and management systems applied.

When discussing utilization of working donkeys, the great majority were found used by adult men for draughting in rural areas. Six to ten years age group was the most used group with intensive work. Therefore, it was found 6-10 years age group was the highest group of helminth infection, due to stress condition as a result of intensive work.

4.6. Conclusion

Donkeys are indispensable and very significant animal for human being all over the world. Although some of draught donkeys' owners were found having a knowledge about management and part of treatment regimes, but the great majority of the respondents were found having problems with animal health care and management. Therefore, there is a high need for extension work among draught donkey's utilizer communities. Also, can be recommended that, the pharmacies must be managed by well-trained veterinarians in order to direct the appropriate drug and the write dose. Furthermore, veterinary clinics should be improved and strengthened with equipments and skilled labors.

Chapter Five

Measurement of prophylactic efficacy of selected drugs

5.1. Introduction

Several reports from Sudan indicated high prevalence rate of helminth in donkeys (Seri *et al*., 2004a; Sawsan *et al*., 2008; Adam *et al*., 2013; Abakar *et al*., 2013; Ismail *et al*., 2016; Gadalkareem *et al*., 2018a). Recent studies reported high prevalence rate of helminth parasites in donkeys in three different districts in Ethiopia (Takele and Nibiret, 2013; Seyoum *et al*., 2015; Molla *et al*., 2015) that are 88.21%, 86.5% and 62.3%, respectively. In donkeys, Doramectin, Ivermectin and Moxidectin injection formulations, as well as Albendazole drench formulation proved to be 100% effective in removing helminth from donkeys (Seri *et al*., 2004b; Seri *et al*., 2005; Sawsan *et al*., 2010; Fangama *et al*., 2013). Recent report indicated reduced efficacy 52-54.29% of Ivermectin against *Parascaris equorum* in France (Laugier *et al*., 2012).

Egg reappearance period (ERP) can be defined as the time from anthelmintic use till parasite eggs can again be detected in the faeces in appropriate quantity that necessitates another treatment. 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 India, the persistent effect of anthelmintics in naturally infected donkeys was reported by Parsani and his colleagues (2011). They indicated that the duration of protection of Fenbendazole and Ivermectin was the same up to six weeks, whereas persistent effect varied from six weeks to twelve weeks in both of the drugs. The objective of this study was to investigate the therapeutic efficacy, persistent effect and treatment intervals of Moxidectin, Ivermectin, Doramectin and Albendazole as an anthelmintic in donkeys harbouring natural worm infestation.

5.2. Materials and methods

5.2.1. Prophylaxis experiment

Ghebaish locality was chosen for the experiment as having easily accessed animals, facilities and suitable management for the performance of this type of experiment. Moreover, the treated animals were left on their environment with their owners with the same feeding system, bedding and housing. Animals were selected on the basis of confirmed infection and willingness of owners.

A total of (39) infected draught donkeys were grouped in to 4 groups for treatments; each group of 10 animals (except group 4 was of 9 donkeys) having different ages and sexes **(Table 5.2.10).** Identification of number of animals per age in each group was done according to capacity of contribution on work.

The animals were kept with their owners working during the day and housed either individually or in groups during night. They were provided with clean water and allowed to feed as in the normal situation either single or (tied) together during day and at evenings **(Appendix 3)**. Each group of donkeys was treated using one type of anthelmintic.

Table 5.2.10: Distribution of donkey treatment groups by age and sex

Group	Total	Sex		Age/sex					
		Male	Female	≤5 years		6-10 years		>10 years	
				Male	Female	Male	Female	Male	Female
A	10	7	3	1	1	4	1	2	1
B	10	7	3	1	1	4	1	2	1
C	10	7	3	1	1	4	1	2	1
D	09	6	3	1	1	3	1	2	1
Total	39	27	12	4	4	15	4	8	4

5.2.1.1. Treatment

Four different formulations of 4 commercially available anthelmintics were used in this study (**Appendix 4)**.

Group 1: Moxidectin paste (Zoetis Manufacturing &Research, Spain) 18.92/g was administered orally at 0.4 mg/ kg body weight.

Group 2: Ivermectin paste (Merial, Brazil) 1.87% was administered orally at 0.2 mg/kg body weight.

Group 3: Doramectin (Montajat Phamaceuticals, Saudi Arabia) 1% w/v was administered subcutaneously at 0.2 mg/kg body weight (1ml / 50 kg body weight).

Group 4: Albendazole (Leads Pharma (Pvt) Ltd (Vet Div) Pakistan) bolus 250 mg was administered orally at 1 bolus (250 mg) /35kg body weight.

Animals' treatment and grouping: The animals were allocated into four treatment groups each of ten animals except the last group comprised only nine animals. The animals in the different treatment groups received treatment as follows:

1. Moxidectin treated group (A) received a single oral dose of Moxidectin paste formulation at the manufacturer's recommended dose of 0.4 mg/kg body weight.
2. Ivermectin treated group (B) received single oral dose of Ivermectin paste formulation at 0.2 mg/kg body weight.
3. Doramectin treated group (C) received single subcutaneous dose of Doramectin injection formulation at the manufacturer's recommended dose of 0.2 mg/kg body weight.
4. Albendazole treated group (D) received single oral dose of Albendazole at 1 bolus 250 mg/35kg body weight equivalent to 7.14 mg/kg body weight.

Then donkeys were monitored for possible adverse or unwanted reactions for 2 hours after administration of each drug.

5.2.1.2. Parasitological investigations

The experiment extended for 105 days. Faecal samples were collected at 0 (before treatment), 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 ……105 days post treatment using the Modified McMaster technique (Anonymous, 1986) with sensitivity of 50 eggs per gram (epg) all faecal samples were obtained per rectum.

Coproculture of pooled samples (before the start of treatment) was conducted, to determine the composition of the nematode population present.

By the end of the experiment the donkey's owners were advised to treat their animals with the available anthelmintics after two weeks. Furthermore, they were informed with the routes of drug administrations and dosing.

5.2.1.3. Data analysis

5.2.1.3.1. Egg re-appearance period (ERP)

The 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 treatment group mean faecal egg count reaches counts of 200 or more epg (arithmetic mean).

5.2.1.3.2. Calculation of efficacy and ERP

The percentage of efficacy of the treatments was calculated from the faecal egg. The efficacy, which was determined by egg count reduction influenced by the treatment usage, was calculated by mean egg count according to the formula (Coles *et al.,* 1992):

$$\text{Efficacy \%} = \frac{\text{mean epg pre−treatment} - \text{mean epg post−treated X 100}}{\text{mean epg pre−treatment}}$$

In order to estimate the time between treatment and the first appearance of the eggs in the faeces, the number of days was individually calculated for each donkey within a group as soon as the threshold is reached (equal or over 200 epg).

5.3. Results

Results obtained in the current investigation are expressed as reduction in the arithmetic egg count for each treatment group at each sampling time point compared to pre-treatment level.

Before treatments on day (0) the coproculture findings showed that the predominant genera were; *Strongylus*, *Dictyocaulus*, *Strongyloides* and *Cyathostomes*. Seven days post treatment all animals in Moxidectin, Ivermectin and Doramectin treated groups, were found negative to eggs of parasites. Efficacy percentage of the four treatment groups used was 100% 14 days post-treatment as in **Table 5.3.11** and **Figure 5.3.10**.

Table 5.3.11: Arithmetic mean (M)± Standard deviation (SD) of epg count and % of efficacy in the different treatment groups up to 98 days post treatment

Days	Moxidectin		Ivermectin		Doramectin		Albendazole	
	M±SD	%	M±SD	%	M±SD	%	M±SD	%
0	1220.00±689.30	-	1140.00±266.50	-	1315±706.70	-	1800.00±1609.00	-
7	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	100.00±212.10	
14	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
21	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
28	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
35	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
42	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0	100
49	0.0±0.0	100	10.00±31.62	99.1	0.0±0.0	100	11.11±22.05	99.4
56	0.0±0.0	100	15.00±47.43	98.7	5.00±15.81	99.6	33.33±55.90	98.2
63	0.0±0.0	100	20±63.25	98.2	10.00±31.62	99.2	77.78±122.80	95.7
70	0.0±0.0	100	35.00±81.82	96.9	30.00±48.30	97.7	94.44±131.0	94.8
77	15.00±47.43	98.8	65.00±88.35	94.3	90.00±93.63	93.2	105.6±126.1	94.2
84	50.00±97.18	95.9	125.00±75.46	89.0	160.00±69.92	87.8	133.3±117.3	92.6
91	105.00±89.60	91.4	205.00±64.33	82.0	225.00±58.93	82.9	211.1±85.80	88.3
98	205.00±79.76	83.2	225.00±33.36	80.3	235.00±41.16	82.1	244.4±39.09	86.4

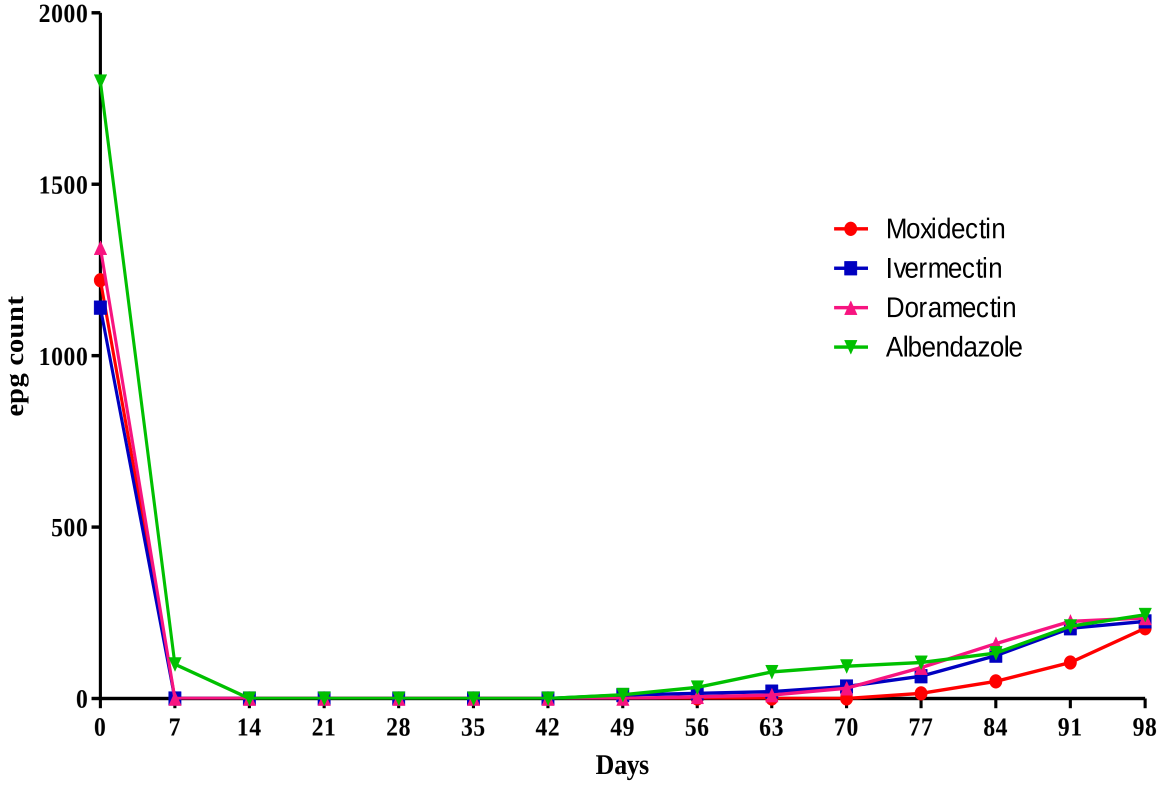


Figure 5.3.10: Arithmetic means overtime of each group

The efficacy of all products, as outlined in the World association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics (Wood *et al*., 1995) is greater than 90% until D98 in Moxidectin treatment and D77 in Ivermectin and Doramectin treatment groups, and D84 in Albendazole treatment group.

Parasite eggs reappeared on day 77 in Moxidectin and on day 49 for Ivermectin and Albendazole treated groups and D56 in Doramectin group (**Table 5.3.11)**. The persistent effect of Moxidectin was continuous up to D 91, while in Ivermectin, Doramectin and Albendazole extended to D 84.

The treatment interval was 98.7±6.96 in Moxidectin, 88.90±8.76 in Ivermectin, 88.20±5.90 in Doramectin and 83.22±14.19 in Albendazole (**Table 5.3.12**). No adverse reactions were observed during the experimental period.

Table 5.3.12: Estimate of the interval from treatment to first reappearance of helminth eggs in faeces

	Mean number of days to reoccurrence of epg>200(± SD [a])			
Products	Moxidectin	Ivermectin	Doramectin	Albendazole
Combined days	98.7±6.96	88.90±8.76	88.20±5.90	83.22±14.19

a Standard deviation

5.4. Discussion

The reduction of faecal egg output is considered as one of the simplest ways for measuring therapeutic efficacy of anthelmintic products. Currently, the FECR test is the standard method to monitor drug efficacy against helminth infection in livestock (Levecke *et al.*, 2012). It is the only test available for quantifying the removal of reproductive adult female worms (Reinemeyer, 2009). In this study, the percentage of efficacy of the products showed no difference between the treated groups up to D77, within a threshold efficacy fixed at 90% as recommended by the guidelines. These results are in line with Seri *et al.*, (2004, 2005) and Sawsan *et al.* (2010). These authors showed efficacy greater than 99% of Ivermectin, Doramectin and Albendazole in donkeys. From D84 onwards, the percentage of efficacy progressively decreased in all treated groups. The small increase of epg output in all groups from D84 onwards should suggest another next treatment. But the overuse of anthelmintics is also undesirable as this may increase selection pressure for resistance (Kelley, 1981).

Therapeutic efficacy of 100% of Moxidectin, Ivermectin, Doramectin and Albendazole, was recorded for all groups on day 14 post treatment. The drugs persistent effect in Moxidectin extended to day 70 post treatment, while in both Ivermectin and Albendazole was extended to day 42 post treatment; while the persistent effect of Doramectin was extended for 49 days. Concerning the impact of baseline FEC, earlier results demonstrated that precision and accuracy of the FECRT improved as levels of egg excretion increased and that FEC method with low analytic sensitivity is not preferred when baseline FEC are low (Levecke *et al.*, 2011). Levecke *et al*. (2012), suggested that the true drug efficacy is likely to be underestimated when FEC are low and drugs are more efficacious, this regardless of the analytic sensitivity of the FEC method and the baseline FEC.

Mercier *et al*. (2001), showed that 100% efficacy was only up to 28 days in horses treated with either combination of Ivermectin 1.87% and Praziquantel 14.03%, Ivermectin 2%, and up to D35 in horses treated with Moxidectin 2%.

In Sudan, donkey owner's tend to tether their animals together during day and/or during night. Helminth infection can easily be transmitted from one animal to the others. Here it is worth mentioning that, the current study was conducted under field conditions where there is no need to add control group so as to monitor the simultaneous variation in epg count. The parasites eggs reappeared and the eggs count reached 200 eggs per gram on day 98 in Moxidectin and day 91 in Ivermectin, Doramectin and Albendazole treated groups.

The results obtained were comparable with that obtained by Entrocasso *et al.* (1996) who compared the efficacy of four Macrocyclic lactones (Ivermectin, Abamectin, Doramectin and Moxidectin) in maintaining reduced faecal egg count in cattle grazing naturally in pasture in Argentina, Brazil and Colombia. They observed reduction in mean cumulative faecal egg count in treated animals 63 days post treatment.

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 interval between the two treatments obtained in the current study is within the range of 10 to eleven weeks. A result would suggest another treatment every 2-3 months. The occurrence of re-infection may be attributed to the fact that encysted cyathostome larvae can survive in the wall of the large intestine is of great practical importance. Sometimes many larvae emerge from the gut wall, causing condition known as larval cyathostomiasis (The Brooke, 2013; Matthews and Burden, 2013). During this condition, egg counts may be negative since the larvae do not produce eggs. The treatment interval recommendation found in this study and based on the reappearance of egg output was roughly 2 months, whatever the product used (Mercier *et al*., 2001).

5.5. Conclusion

The present study provided a comprehensive description of Moxidectin and Ivermectin, Doramectin and Albendazole efficacy, persistent effect, and treatment interval in Western Kordofan State.

The therapeutic efficacy percentage of the four treatment groups used was 100% 14 days post-treatment. The persistent effect of Moxidectin was continuous up to D 91, while in Ivermectin, Doramectin and Albendazole extended to D 84.

The treatment interval was 98.7±6.96 in Moxidectin, 88.90±8.76 in Ivermectin, 88.20±5.90 in Doramectin and 83.22±14.19 in Albendazole.

Chapter Six

General discussion

West Kordofan State is the home of a large population of equines. Nevertheless, there are no investigations or data on equine diseases prevalence particularly parasitic, or plans for preventive measures especially in donkeys. To plan proper animal health improvement strategies, especially draught donkeys as neglected animals, it is essential to assess the parasitic diseases status and their influence on these draught animals population. It is also significant to map the prevalence of different helminth infections to help in defining their geographical distribution and to assess the impact of risk factors and their effect on the epidemiology of these diseases.

This study was conducted to: assess the prevalence rates of helminthic infestation, its associated risk factors and to investigate prophylactic measurements for the control of helminth infections in draught donkeys in West Kordofan State. Also, to come out with recommendations that can contribute to designing and promoting sustainable control practices of gastrointestinal helminth in draught donkeys in the study area. Prevalence among age groups and in the three main seasons was estimated. The perception of owners about their working donkey's diseases, their knowledge, management practices, utilization and environmental factors were also investigated. The general results confirmed the presence of helminth parasites which raise alarm for a possible future hazard.

Donkeys need more attention to apply strategic programmes for controlling gastro-intestinal nematodes what makes donkeys more healthy and productive (Mohmmed, 2009). Also, continuous awareness creations to donkey owners on proper management and handling should be in place in order to enhance the impact of strategic mass deworming (Berihun, 2015).The field veterinarians should raise the donkey owners awareness on improving the housing and feeding management system and to providing sufficient food and shelter. More prevalence and efficient treatment and regular deworming program should be implemented using the available broad spectrum anthelmintics (Wubie and Getaneh, 2015; Molla *et al.*, 2015; Mohammed *et al*., 2016; Belay *et al*., 2016; Gebreyohans *et al*., 2017).

Donkeys are very effective and crucial animals in livelihood practices. In the study area, there were no faecal testing, no strategic treatment, absence of public awareness and preventive measures. The majority of working donkey's owners use the drugs randomly without a clear plan. Moreover, the veterinary institutions in the area were deteriorated due to poor infrastructure, lack of skillful manpower and limited laboratory facilities and financial support. This situation, may lead to decline in the general production and lessen the donkey's contribution in socio-economic life because of insufficient health care and welfare practice.

To the best of our knowledge this is the first study about the assessment of parasitic infections among draught donkeys in this area. The overall prevalence rate was found (38.25%) and prevalence rates in *Ghebaish* and *Alfula* were (20.25%) and (18%), respectively. This can be related to the difference in environment and management patterns between the two areas. The overall prevalence in this study was found lower than other studies: Seri *et al*. (2004); Najat (2006); Mezgebu *et al*. (2013); Tsegaye and Chala (2015); Wako *et al*. (2016); Belay *et al*. (2016); Mohammed *et al*. (2016); Abdurhman *et al*. (2016); Abdulahi *et al*. (2017); Kassa and Zeleke (2017). This may be attributed to differences in management systems, environmental conditions and numbers of animals examined. There were 1200 donkeys were examined in this study.

In the present study spectrum of helminth were identified with variable infection intensity. The most common encountered parasites were *Strongylus spp*, which represented about 59.91% of the infections, followed by *Dictyocaulus*, *Strongyloids*, *Cyathostomes* and *Oxyuris* with rates of (6.54%), (5.01%), (3.49%) and (3.49%), respectively. The Other parasites including *Parascaris*, *Anoplocephla*, *Paranoplocephla* and *Trichostrongylus* were found in low proportions in this study.

Many studies have shown that *Strongylus* usually affects young animals mostly horses and donkeys (Duncan and Pirie, 1975). In Sudan, gastrointestinal parasites, especially *strongylids*, are commonly present among donkeys. The prevalence of these nematodes among equines depends on many factors including environmental and breeding-related conditions (Romaniuk *et al*., 2007; Kornaś *et al*., 2008). The most significant pathology of *large strongyles* is caused by migration of the larval stage through the cranial mesenteric arteries and adjacent branches (Matthews and Burden, 2013; Khan *et al.*, 2015). Pathogenesis results in fatal consequences sometimes. These are considered to be precursors of damage to organs of the digestive tract and severe disorders in enzymatic and hormonal processes (Assis and Araújo, 2003). Nevertheless, Anthelmintic treatment strategies designed to control *Strongylus* have been extremely successful in reducing prevalence, morbidity, and mortality from this parasite (Kaplan, 2002).

Dictyocaulus was identified in 6.54% of the working donkeys in the present study, which was reported for the first time in Sudanese donkeys by Seri *et al*. (2004). *Dictyocaulus arnfieldi* and its association with donkeys was also reported in several studies: Clayton and Neave (1979); El-Seify *et al.* (2010); Veneziano *et al*. (2011); Matthews and Burden (2013); Feye and Bekele (2016). Lungworm (*Dictyocaulus arnfieldi*) can be present in large numbers in donkeys without the animals showing any signs. However, donkeys can pass on the infection to horses, which might suffer from lung problems resulting in coughing and discharge from the nostrils (Svendsen, 2015). Consequently it is a dangerous hazard for donkeys and horses in Sudan.

In this study *Strongyloids* were found in (5.01%) of donkeys studied. Rajmund *et al*. (2015) stated that; Young animals and foals can be also infected with *Parascaris equorum* and *Strongyloides westeri*. In addition, in Europe and

America certain helminth, such as *Parascaris equorum* and *Strongyloides westeri*, are thought to cause clinical disease only in young animals, as adults develop resistance (The Brooke, 2013). Persistence of *strongyloides* in the environment presents a danger for infection of susceptible animals even after long period.

In the current study, *small strongyles* (*Cyathostomes*), as well as *Oxyuris* were found in (3.49%) of the donkeys. *Small strongyles* are the most common internal parasite in adult equines have non-migratory life cycle and occur frequently in foals. Larvae penetrate the walls of the intestines, where they encyst. During this stage they cause colic, bleeding, anemia, protein loss, and intestinal mal-absorption. In the spring (and during times of stress), larvae rapidly emerge from the gut wall and cause severe diarrhea (verminous enteritis), chronic weight loss, and unthrifty appearance (Gore *et al*., 2008; The Brooke, 2013). These pathological and clinical effects might have been occurring unnoticed. Faecal examination in donkey and equines in general is not done in colic. This deduced that faecal examination should be routinely done in cases of intestinal complaints.

Pinworms (*Oxyuris equi*) are common parasites in equids. Although they are often non-pathogenic, but they can cause intense pruritus around the anus which can lead to self-trauma (The Brooke, 2013).

In this study with respect to seasonal distribution of parasitic infections, most of infections were found during dry hot season representing (51.63%) followed by wet hot and dry cool constituting (24.84%) and (23.53%), respectively. However, relatively different findings have been reported from Sudan; Seri *et al*. (2004) found slightly different rates in the cold season which he thought encouraged higher incidence of gastrointestinal nematodes (72.5%), more so than hot (69.3%) and rainy seasons (68.5%). Whereas Abdurhman *et al*. (2016) found wet hot season had higher mean parasites count (5411.5 ± 1694.4) in comparison with hot dry (1795.9 ± 399.6) and cool dry (1719.9 ± 522.4) seasons. This may be due to the difference in climate in the study areas; these authors conducted their studies in much wetter climates than this study. As well as the number of animals included was smaller than in this study.

In this study findings the prevalence of *Strongylus spp* and mixed infection were found to be significantly higher during the dry hot season in the two localities. The severity of helminth infections is affected by the environment, where the general animals' health improves during the wet season as compared to the dry hot season, when the vegetation condition is poor which predisposes to more pathogenic effect of GIT parasitic infection. As in this study; the infected animals were found higher in poor vegetation, during dry hot season. This is agreeing with Stevenson (2005); Wubie and Getaneh (2015).

For seasonal appearance of some parasites in this study, *Cyathostomes* appeared only during wet season. This can be attributed to the fact mentioned by (Stevenson, 2005); environmental factors include aspects of climate (temperature, humidity, rainfall) as well as aspects of animal management (management of animals in a certain area of a country may result in high rates of disease that may not be seen in other areas). Other researchers mentioned that; *Cyathostomins* are highly prevalent and potentially pathogenic parasitic nematodes found in the large intestine of horses and other equids worldwide. And may rarely cause cyathostominosis or colitis; however signs and symptoms of both can vary significantly (Mcarthur *et al*., 2015; Burden and Getachew, 2016).

As severity of helminth infection is concerned, in this study the infection was found representing (68.85%) for mild, (17.86%) for severe and (13.29%) for moderate infection. The overall mean egg per gram count (epgc) was 704.14±116.59 with a range of 50-10200 (epg). The highest egg per gram count was found in dry hot season. Approximately similar results were found by Sawsan (2009) who found the severity of infection showed 81.25% for mild, 7.89% for moderate and 10.86% for severe infection. Arithmetic mean of egg per gram of faeces (epg) count was 750.14±1071.95 with range of (50-11800 epg) and the highest range reported was in April.

Infections can be determined by many risk factors. Intrinsic determinants (factors) related to the host which can influence the frequency of occurrence of infection and disease are; species, breed, age and sex. Extrinsic determinants of disease are also important in epidemiology in that they can have effects on the host, the agent, and on the interactions between the host and the agent. There are three major extrinsic determinants; which are climate, soils and man (Putt *et al*., 1988).

Animals reared under a good management system tend to possess strong immunity and demonstrate resistance to diseases. Body condition score was significantly associated with infection rates. Donkeys with poor body condition score were significantly associated with high parasitic activities in their gastrointestinal tracts depriving them of adequate absorption and assimilation of digested nutrients resulting in emaciation and cachexia (Mohammed *et al*., 2016).

In this study, when the environmental factors and vegetation were considered, the majority of infections were found in poor vegetation. These results appeared matching the results of the seasonal distribution of infections, where most of the infections were found during dry hot season where there was poor vegetation which acts as risk factor that predisposes to parasitic infection.

Feeding strategies for the wet season are described, as well as the nutritional characteristics of the main forages and supplements used by the animals' owners. It was established that grazing on native grassland could afford sufficient energy and protein for donkeys at maintenance level and for those performing only a moderate amount of work. Nonetheless for those with heavy work they may require collection of food which might increase the risk of infection (Colunga *et al*., 2005). Whilst in places where donkeys are used for work, good management generally involves trying to ensure the animals eat enough to meet their daily requirements for food. This can be difficult where there are large seasonal fluctuations in the quantity and quality of forage available (Pearson and Mohammed, 2000).

In this study, it was found that, the infections were found higher mainly in moderate and bad body condition animals, when comparing the positive results with negative results. High infection rates were found in animals fed indoor.

In the current study the majority of the infected animals were kept tethered in housing with sandy bedding. These factors may increase the risk of animal infection due to difficulties in cleaning and may act as suitable environment for development of infective stages. These results agreed with (Gadalkareem *et al*., 2018b). These findings also agree with Magzoub (2002) and Polley (2010) where they mentioned; hosts, parasites and the local environment were closely related. The structure and type of the grass depend upon soil type, which determine the degree of formation of the (mat) between the soil and the grass that keeps the high humidity for weeks in dry seasons, which decreases the change of temperature, and helps in presence and growth of larvae of parasites.

Equines are particularly prone to parasitic infections during grazing on a pasture. If the pasture is not regularly cleaned from manure, it can easily become a reservoir of increasing number of infective larvae (Rajmund *et al*., 2015). The present study showed that the frequency of disposal of manure was weekly for the majority of the infected animals. Thus, daily cleaning was appropriate for better prevention. These results were found in complete agreement with Gadalkareem *et al*. (2018**b**).

In the present study, the great majority of the infected donkeys were found related to the owners who were found ignorant about the presence of helminth infections and the use of anthelmintic treatments, and had no clear idea about the response of the treatment. Furthermore, regarding the sources of treatment, the highest infection rates were found in animals of owners who had no idea of the source with a rate (58.87%) followed by from pharmacy (33.77%), from clinic

(04.33%) and from friend (03.03%). This means that a lot of animal owners were ignorant about treatment regimes and were not supported with full information about anthelmintic treatments from veterinary service delivery systems.

Hard work and physiological stress may result in immune-compromise (The Brooke, 2013). Consequently, diseases infection rates may increase. For utilization of draught donkeys in this study, the great majority were found used by adult men for carts draughting in rural areas. Animal age group of 6-10 years was the most used group with intensive work. Therefore, it was found the 6-10 years age group had the highest group of helminth infection, due to stress condition as a result of intensive work. Therefore, there is urgency for a complete plan in improving the health and welfare status of draught donkeys, involving community based awareness creation joined with placing access to reliable veterinary service to working equines. Regular and strategic deworming programmes with efficacious anthelminthics should be carried out (Wubie and Getaneh, 2015; Berihun, 2015; Belay *et al*., 2016; Mohammed *et al*., 2016; Gebreyohans *et al*., 2017; Gadalkareem *et al*., 2018**b**).

The reduction of faecal egg output is considered as one of the simplest ways for measuring therapeutic efficacy of anthelmintic products. Now, the only method available for determining if anthelmintics are effective on a farm is the faecal egg count reduction test (FECRT) in which faecal egg counts are measured both before and 14 days after treatment (Kaplan and Nielsen, 2010; Levecke *et al*., 2012).

In this study, the percentage of efficacy of the products showed no difference between the treated groups up to D77, within a threshold efficacy fixed at 90% as recommended by the guidelines. These results are similar to with Seri *et al.* (2004, 2005) and Sawsan *et al.* (2010).

The current study showed that; therapeutic efficacy of 100% of Moxidectin, Ivermectin, Doramectin and Albendazole, was recorded for all groups on day 14 post treatment. The drugs persistent effect in Moxidectin extended to day 70 post treatment, while in both Ivermectin and Albendazole was extended to day 42 post treatment; while the persistent effect of Doramectin was extended for 49 days.

Regarding the impact of baseline FEC, earlier results demonstrated that precision and accuracy of the FECRT improved as levels of egg excretion increased and that FEC method with low analytic sensitivity is not preferred when baseline FEC are low (Levecke *et al.,* 2011). Moreover, Levecke *et al*. (2012), suggested that the true drug efficacy is likely to be underestimated when FEC are low and drugs are more efficacious, this regardless of the analytic sensitivity of the FEC method and the baseline FEC.

In Sudan and in West Kordofan in particular, donkey owners tend to tether their animals together during day and/or during night. Helminth infection can easily be transmitted from one animal to the others. It worth mentioning that, the current study was conducted under field conditions where there is no need to add control group so as to monitor the simultaneous variation in epg count. The parasites eggs reappeared and the eggs count reached 200 eggs per gram on day 98 in Moxidectin and day 91 in Ivermectin, Doramectin and Albendazole treated groups.

The results found in this study were similar to those obtained by Entrocasso *et al.* (1996) who compared the efficacy of four macrocyclic lactones (Ivermectin, Abamectin, Doramectin and Moxidectin) in maintaining reduced faecal egg count in cattle grazing naturally in pasture in Argentina, Brazil and Colombia. They observed reduction in mean cumulative faecal egg count in treated animals 63 days post treatment.

This study showed that; the efficacy of Ivermectin was 100% on day 7, the faecal eggs count reduction, which 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 that agree to finding in horses reported by Costa *et al*. (1998).

The interval between the two treatments obtained in the current study is within the range of ten to eleven weeks. A result would suggest another treatment every 2-3 months. The occurrence of re-infection may be attributed to the fact that encysted *Cyathostome* larvae can survive in the wall of the large intestine is of great practical importance. Sometimes many larvae emerge from the gut wall, causing condition known as larval Cyathostomiasis. During this condition, egg counts may be negative since the larvae do not produce eggs. The treatment interval recommendation found in this study and based on the reappearance of egg output was roughly 2 months, whatever the product used (Mercier *et al*., 2001).

Another aspect is the possible resistance to anthelmintics. Anthelminthic resistance of small *strongyles* is well documented (Wolf *et al*., 2014). Drug resistance in equine gastro-intestinal parasitic nematodes has been reported throughout the world. While the focus is usually put on *Cyathostomins*, observations of Macrocylic lactone failure against *Oxyuris equi* have accumulated over the last decade (Sallé *et al*., 2016).

Conclusion

The study revealed that donkeys in the study area were infected with a variety of helminth, which were representatives of the important equine pathogenic parasites found in Sudan.

There is a high need for extension work among draught donkeys utilizing communities. Therefore, veterinary medical institutions should be managed by well-trained veterinarians in order to deliver awareness services, and to direct animal owners towards the appropriate drug with the right dose. The veterinarians should also be expert in communication and transfer of knowledge to overcome unawareness for better practices. Furthermore, veterinary clinics should be improved and strengthened with equipments and skilled cadre. For clean donkey bedding environment, it's better to follow frequent cleaning pattern.

The comparative prophylactic effect and different preparations proved the efficacy and importance of this type of studies to control helminth.

Recommendations

Further studies to be conducted on the epidemiology and control of helminthiasis in donkeys in the whole country in the context of donkey research project.

More drugs are to be tested as alternative treatment and prophylactic as step for the registration as routine drugs.

Establishment of standard protocols for detection of internal helminth includes blood examination.

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Appendices

Appendix 1: List of materials, kits and equipments used in the study

(A)List of materials used:

1. Flotation fluid

Saturated salt solution	
Sodium chloride (kitchen salt)	400 g
Water	1000 ml
Specific gravity	1.200

2. (10%) Formaldehyde.
3. Distilled water.

(B) List of kits and equipments:

1. McMaster's microscope slides (two chambers with gridlines).
2. Heparinized vacutainers (5ml).
3. Sensitive scale.
4. Cold chain facilities from the field to the laboratory.
5. Microhaematocrite reader, centrifuge and Capillary tubes.
6. Compound microscope (Labomed, Labo America, USA)
7. Miscellaneous plastic and glass ware for faecal and blood sample collection.

Appendix 2: Donkey owners' information

Sudan university of Science and Technology

College of postgraduate Studies

Questionnaire

- ✓ هذا البحث لأغراض الدراسة العلمية فقط
- ✓ المعلومات الواردة في هذا الإستبيان سرية ولإستخدام الدارس فقط

Investigation of working donkey helminth parasites in West Kordofan State

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1. Personal data

Date .... **code**...

Animal owner.. **Locality**...

Contact: Tel.............................location...

2. Risk factors

A. Animal factors

Animal age (years): …................... **Sex:** Male ( ) Female ( )

Donkey type: Hur ( ) Diplawi ( ) Mixed ( )

Body condition score: Good ( ) moderate ( ) bad ( )

Faecal consistency: Normal ( ) soft ( ) diarrhoea ( )

Comments: ....

………………………………………………………………………………………..

B. Management factors:

Feeding type: Grass ( ) supplementation ( )

Feeding method: Alone ( ) group feeding ( )

Feeding habits & water: Indoor ( ) outdoor ( ) if indoor

Time**:** Day time:………… Hours, Night …………….. Hours

Housing: Zariba ( ) tethered ( ) free ( )

Bedding: Sand ( ) hay or straw ( ) Not available ( )

Disposal of manure: No ( ) Yes ( ), if yes frequency

monthly ( ), weekly ( ), specific time ( )

Presence of helminth: I Know ( ) don't Know ( )

Use of anti-helmintics: Yes ( ) No ( )

Response: Cured ( ), No change ( )

Source of anti-helmintics: Pharmacy ( ), Clinic ( ), Friend ( )

Presence other infections: Yes ( ), No ( ) **if yes,** name………………………………………………………………………………

Type of treatment: …………………………………………………………………

Comments: ....

…………………………………………………………………………………**.**

3. Environmental factors:

Dry hot months vegetation: Good ( ), poor ( )

Wet cool months vegetation: Good ( ), poor ( )

Wet hot months vegetation: Good ( ), poor ( )

4. Utilization:

Work intensity: Low ( ) Moderate ( ) intensive ( )

Type of work: draught ( ) Riding ( ) Multiple ( )

Donkeys user: Adult owner ( ) Child ( ) Women ( ) More than one ( )

Donkey usage: Urban ( ) Rural ( )

Comments: ....

…………………………………………………………………………………
…………………………………………………………………………………
…………………………………………………………………………………

Appendix 3: Donkeys tethered together at a market during the study

Appendix 4: Anthelmintic drugs used in the clinical trial

No.	Trade name	Generic name	Composition	Manufacturer
1	Eqvalan	Ivermectin paste 1.87%	Ivermectin 1.87%	Merial, **Brazil**
2	Equest	Moxidectin Paste 18.92mg/g	Moxidectin18.92mg/g	Zoetis Manufacturing &Research, **Spain**
3	Doramec	Doramectin 1%w/v	Doramectin 10mg Butylated Hydroxytoluene (Antioxidant) 0.1%w/v	Montajat Phamaceuticals, **Saudi Arabia**
4	Vety alben bolus	Albendazole bolus	Albendazole USP 250mg	Leads Pharma (Pvt) Ltd (Vet Div) **Pakistan**

Appendix 5: Donkeys used for cart draught and water fetching

Appendix 6: Donkeys used as a means of transport in the rural areas

Appendix 7: Heavy presence of donkeys in the rural communities for different uses

Appendix 8: Multipurpose utilization of donkeys by women in carts draughting at Ghebaish town

Appendix 9: Sudan States

West Kordofan State

Available at: https://www.google.com/search?q=Sudan+States+maps

Appendix 10

Sudan: West Kordofan State Administrative Map (January 2015)