EFFICACY OF ALBENDAZOLE AGAINST EXPERIMENTAL INFECTIONS OF COENURUS CEREBRALIS IN GOATS. HUMORAL AND MOLECULAR CHARACTERIZATION OF COENURUS CEREBRALIS

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Universitat Autònoma de Barcelona

Doctoral Thesis

Departament de Farmacologia, Terapèutica y de Toxicologia

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Doctoral Thesis submitted by **Sonia Maria de Santana Afonso** as partial fulfilment of the requirements for degree of Doctor by Universitata Autònoma de Barcelona. The studies included in the thesis have been realized under the direction of **Professor Margarita Arboix Arzo** from "*Departamento de Farmacología, Terapéutica y de Toxicología*", Bellaterra, Spain and **Professor Luís Carlos Bernardo Gil das Neves** from Department of Veterinary Tropical Diseases, Faculty Sciences, University of Pretoria. Doctorat Program of Universitat Autònoma de Barcelona".

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CERTIFIQUEN:

Que la memòria titulada "**EFFICACY OF ALBENDAZOLE AGAINST EXPERIMENTAL INFECTIONS OF *COENURUS CEREBRALIS* IN GOATS. HUMORAL AND MOLECULAR CHARACTERIZATION OF *COENURUS CEREBRALIS"***, presentada per **Sonia Maria de Santana Afonso** per optar al grau de Doctor, ha estat realitzada sota la seva dirección i, considetant-la finalitzada, autorizen la seva presentació per que sigu avaluada pel tribunal corresponent.

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Bellaterra, 9 de Deciembre de 2015

Professora Margarita Arboix Arzo **Professor Luis Carlos Bernardo Gil das Neves**

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Abbreviations

ABZ: Albendazole

ABZSO: Albendazole sulphoxide

$ABZSO_2$: Albendazole sulphona

AE: Alveolar echinococcosis

AgF fluid: *Taenia multiceps* antigen from cyst fluid

AgFE: *Taenia multiceps* antigen from membrane and scolex

AgO: *Taenia multiceps* antigen from oncosphere

ANOVA: Analysis of variance

AUC: Area under the plasma concentration time curve

AUMC: first moment curve

BZD: Benzimidazole

CE: Cystic echinococcosis

Cmax: Peak drug plasma concentration

COI: Cytochrome c subunit 1 ()

Cp • t : Concentration times time

CYP: Cytochrome P-450

DD: Gel double diffusion

DNA: Deoxyribonucleic acid

ELISA: Enzyme linked immune assay

FMO: Flavine monoxygenase

GA: Albendazole matrix tablet

GCP: Good clinical practice

HPLC: High Performance Liquid Chromatography

HP-β-CD: Hydroxypropyl-β-cyclodextrin

IB: Immunobloting

IEP: Immunoelectrophoresis

IEP: Immunoelectrophoresis

IgA: Immunoglobulin A

IgE: Immunoglobulin E

IgG: immunoglobulin G

IgM: Immunoglobulin M

IHA: Indirect haemaglutination antibody

IIA: indirect immunoflurescence antibody

ITS2: The second internal transcribed spacer of ribosomal

KA: Albendazole-β-cyclodextrin

LDH: Malate dehydrogenase

MRT: Mean residence time

NCC: Neurocysticercosis

ND1: NDH dehydrogenase

NTB: Netobimin

OXB: Oxibendazole

PBS: Phosphate buffer saline

PCR: Polymerase chain reaction

PCR-RFLP: Polymerase chain reaction-based restriction fragment length polymorphism

RIA: Radioimmunoassay

SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SSCP: Single-strand conformation polymorphism

$\mathbf{t_{1/2}}$: Elimination half-life.

TBS: Tris Buffered Saline

Th: *Taenia hydatigena* antigen from membrane and scolex

Tmax: Time to peak plasma concentration

Ts*: Taenia solium* antigen from membrane and scolex

UNICEF: The United Nations Children's Fund

WHO: World Health Organization

ABSTRACT

Efficacy of Albendazole Against Experimental Infections of *Coenurus Cerebralis* in Goats. Humoral and Molecular Characterization of *Coenurus Cerebralis*

In Mozambique, goats are an important protein source and a major form of income for rural families. Goats are seriously affected by parasitic infections which are a major cause of morbidity and mortality and are considered to be an important constraint to goat production. One of these parasitic diseases is coenurosis, caused by *Coenurus cerebralis*, the larval stage of *Taenia multiceps*. Coenurosis is a common disease of the central nervous system of ruminants, rarely found in humans. There are no reports on the molecular characterization of *T. multiceps* from Africa. The use of albendazole (ABZ) could be an alternative for *C. cerebralis* treatment. Understanding the factors related to the pharmacokinetic behaviour of albendazole in goats is very important to maximize the broad-spectrum anthelmintic activity. In this context, the study focused on the parasite and albendazole in goats. Regarding *Taenia multiceps*, aspects related to its occurrence in natural conditions and the morphological and molecular characteristics in goats were studied. Moreover, the pharmacokinetics of albendazole in simple and multiple doses in goats that were healthy and infected with *T. multiceps* were described and the efficacy of albendazole in those experimental infections was assessed.

A high prevalence of different parasites was found in goats slaughtered at Tete Municipal Abattoir. Prevalence, infection rates, cyst localization and size at natural and experimental infection of *Coenurus cerebralis* in goats were described. The molecular characterization showed sequences of *Taenia multiceps* and no genetic differences were found between cysts with cerebral and non-cerebral localization. The immunochemical results of this study on antibody and antigens corroborate the suggestions from previous reports on the difficulty of developing immunoassays of practical use based on crude or sodium dodecyl sulphate polyacrylamide gel electrophoresis fractioned antigens.

The plasma kinetic profile of ABZ and its metabolites after single or multiple oral administration of ABZ in healthy goats of different ages and sex and *T. multiceps*-infected adult males were analyzed, compared and discussed. Additionally, infected animals were treated with a single oral dose of 10 mg/kg ABZ. Twelve hours after drug administration, muscles, brain and cyst fluid

were collected. Plasma, muscles, brain and cyst fluid were analysed using HPLC method. In healthy goats, age differences in the plasma profiles of ABZ metabolites were observed but no gender related differences were observed. The pharmacokinetic parameters of ABZ metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphona ($ABZSO_2$), obtained from infected and healthy goats treated with 10 or 20 mg/kg of ABZ, showed statistically significant differences in concentration (Cmax), time of peak plasma concentration (Tmax), area under the curve (AUC) and mean residence time (MRT). Lower Cmax and AUC for both metabolites were observed in infected goats when compared with healthy goats treated with the same dose, (10 mg/kg of ABZ). This suggests that *T. multiceps* cysts cause pathophysiological changes that alter the kinetics of ABZ and its metabolites. In a multiple ABZ dose (3x10mg/kg), ABZSO appeared earlier and in higher concentrations than $ABZSO_2$ in the first 24 h, but after 48 hours, the plasma concentrations of $ABZSO_2$ were greater than ABZSO. This suggest that ABZ can induce the ABZSO metabolism to $ABZSO_2$

In a controlled trial with experimental *T. multiceps*-infected goats, two months after infection, the efficacy of ABZ was 90.3%, 72.7% and 73.9% for 10mg/kg/3 days, single 10mg/kg and 20mg/kg doses, respectively. No differences were observed in cyst viability between treated and control groups for cysts, 5 months after infection. The results of this study indicate that ABZ is effective, in goats, against cysts of *T. multiceps* located in tissues outside the brain two months after infection.

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INTRODUCTION

1. Introduction

1.1. *Taenia multiceps*

1.1.1. *Taenia multiceps* general biology

The cestode *Taenia multiceps* (*Leske, 1780*) (= *Multiceps multiceps*) belongs to the family Taeniidae (*Ludwig, 1886*) class Eucestoda. The adult stage of this parasite lives in the small intestine of dogs, foxes, coyotes and jackals, which are definitive hosts of *T. multiceps*. The infection by *T. multiceps* larva is known as coenurosis (gid or sturdy), characterized by the development of cysts in the brain and others tissues of the intermediate host. It is a nervous system disease which occurs mainly in sheep. Other species such as goats, cattle, camels, gazelles, horses, buffalo, wild ruminants and monkeys can also be affected (Sharmaa and Chauhanb, 2006). Humans may be infected accidentally as an intermediate hosts; the development of larva takes place in the central nervous system or in subcutaneous or intramuscular tissues (Ing et al., 1998).

The life cycle of this parasite is indirect, requiring one intermediate host to complete its development. In the definitive host (dog and wild carnivores), mature proglotid segments are liberated from the body of the tapeworm and eggs are discharged into the environment with the faeces. Within the eggs are oncospheres, or hexacanth (six-hooked) embryos. Eggs are surrounded by hundreds of tiny blocks held together with cement, this is called the eggshell (Smyth, 1994). A susceptible intermediate host ingests T. *multiceps* eggs with contaminated food or water and by the action of digestive enzymes in the small intestine, the eggshell is dissolved and the oncosphere is released. The oncosphere penetrates the intestinal wall with the help of its six embryonic hooks and migrates through the circulatory and lymphatic system to the predilection sites (Soulsby, 1982; Smyth, 1994). Coenurus cysts develop in internal tissues and organs, the central nervous system and the spinal cord being the most frequent locations for *T. multiceps* larva (*Coenurus cerebralis*) in sheep. In goats, cysts are commonly reported in subcutaneous and muscular tissues, as well as in other organs (Soulsby, 1982; Sharmaa and Cauhand, 2006). The fluid-cyst is infective to the definitive host after a period of six to eight months (Soulsby, 1982; Hago and Abu-samara, 1980; Noorddin et al., 1996; Urquhart et al., 1996; Vink et al., 1998; Achenef et al, 1999). The larval fluid-filled cyst possesses many scolices that remain attached to the internal surface of the cyst membrane (*multiceps*: multi = many, ceps = head, referring to the scolices) (Soulsby, 1982). The cyst is filled with a transudate fluid that originates in host tissues. In tissues other

than the brain, this cyst is surrounded by a hyaline, adventitious capsule of host origin (Smyth, 1994). In cattle, cyst diameters vary from 2 to 6 cm, while in sheep they range from 0.5 to 7 cm (Achenef et al., 1999; Biyikoglu et al, 2001; Ozmen et al., 2005; Scala and Varcasia, 2006). The goat is the species harbouring bigger cysts, there are reports of cysts up to 8 cm in diameter (Noorddin et al., 1996; Sharma et al., 1998; Vink et al., 1998). The biggest reported cyst (26 x 31 cm) was found on a goat kidney (Hago and Abu-Samara, 1980). The life cycle of the parasite is completed when flesh containing mature coenurus cysts is consumed by a definitive host (dogs and wild carnivores). Once freed from the intermediate host tissue by digestion in the small intestine of the definitive host, scolices of coenurus cysts evaginate, attach to the gut wall using the suckers and rostellar hooks and develop into dult tapeworms. The prepatent period in dogs is between 38 and 43 days, adult worms grow up to 100 cm in length (Willis and Herbert, 1987; Kimberling, 1988; Kaufman, 1996).

Diagram illustrating the life cycle is presented in figure 1.

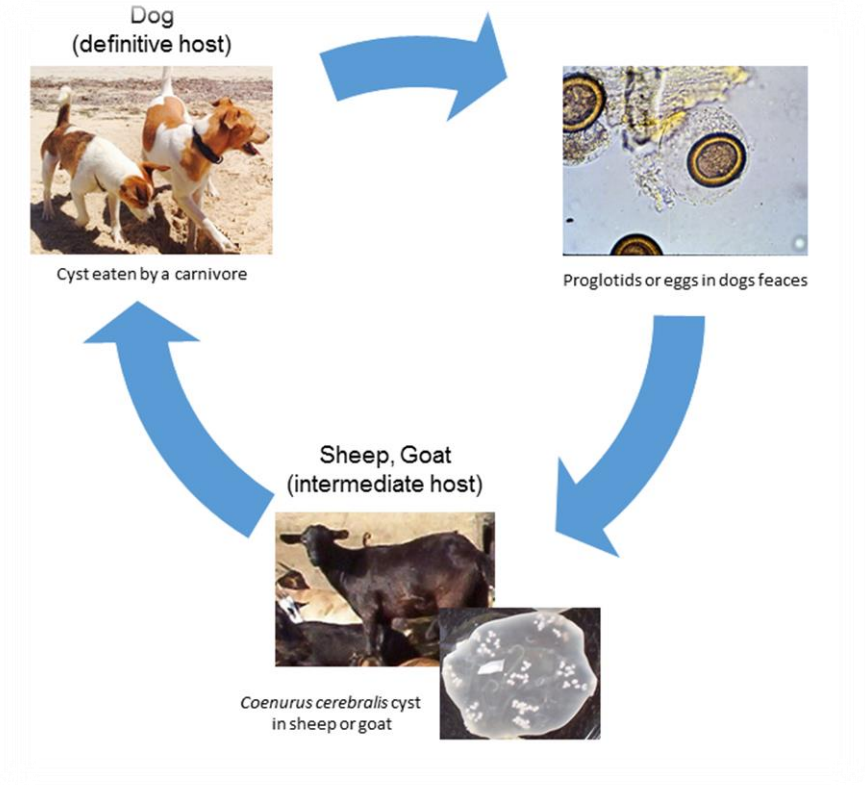

Figure 1- Life cycle of *Taenia multiceps*

1.1.2. Distribution of *Taenia multiceps* larva

Coenuoris is distributed worldwide, and it is responsible for high rates of mortality and morbidity in small ruminants, causing great economic losses, mainly in developing countries in Africa and southwestern Asia (Achenef at al., 1999; Wu et al., 2012). Despite the few studies conducteded in Africa, there are estimates that coenurosis presents a serious threat to nearly three-quarters of the population of small ruminants in this continent (Sharma and Chauhan, 2006). In Kenya, coenurosis is ranked as an important cause of mortality (Kagira and Kanyari, 2001).

Studies to determine the prevalence of coenurosis in small ruminants show high variability in their results. This variation could be attributed to numerous factors, such as the nature of the production system, the type of husbandry, the presence and abundance of dogs or other intermediate hosts, the practice of deworming, the characteristics of the slaughter process and the feeding habits of carnivores. Besides the above mentioned aspects mainly related to the hosts, sociological and economic factors, geographic location and ecological diversity, also play an important role in the epidemiology of *T. multiceps* (Sharma and Chauhan, 2006).

The wide range of prevalence values is documented in studies conducted in different countries and regions of the world. In Tanzania a *Coenurus cerebralis* prevalence of 44.4% in small ruminants (45.6 % in sheep and 43.3 % in goats) was determined (Miran 2013; Miran et al., 2015), while in Ethiopia, between 1992 and 2015, the levels of prevalence of *C. cerebralis* ranged between 2.3% and 37.4% (Achenef et al., 1999; Deressa et al., 2012; Adane et al., 2015). Surveys carried out in southwestern Asia and in the north of Africa indicate prevalence values in sheep of 16.35%, 0.09 % -18.65% and 18.3% for Turkey, Iran and Egypt respectively(Uslu and Guclu, 2007;Oryan et al., 2010; Tavassoli et al., 2011; Oryan et al., 2012) .

In Mozambique, the prevalence of coenurosis is unknown; there are non-published records, mainly from slaughterhouses, but no systematic data on the occurrence of the disease is available in the country. The first published report on *T. multiceps* larva infection in goats was presented in 1998 (Vink et al., 1998): from 130 inspected goats at Tete abattoir, 18 (13.8%) had at least one cyst in the brain and from those, only 2 (1.5%) goats presented nervous symptoms. Furthermore, 8 (6%) of the goats showed *Coenurus* cysts in the muscles (Vink et al., 1998). In another survey, Atanasio (2000) found 8% and 7.3% of the goats

infected with larva of *T. multiceps* in Gaza and Tete provinces respectively There is no data related to the occurrence of *T. multiceps* larva in sheep.

Prevalence rates, generally have higher values in young animals than in adults, which in many cases means a substantial economic loss (Scala and Varcasia, 2006; Adane et al., 2015). Ozmen et al. (2005) observed that among infected animals, 72% were between 6 and 24 months old (average: 19.3 months). This observation is supported by the work of Gicik et al. (2007) in which they demonstrate that 15% of the infected one year old animal. This propotion increased to 21.7 % at 2 years in contrast with 11.4 % found in adult animals. In a very similar study, Tavassoli et al. (2011), indicate a very similar pattern of variation in prevalence rates with age, where a prevalence of 42.02 % was found in animals between 0.5 and 2 years, 22.50% in animals between 2 and 4 years and 8.92% in animals over 4 years. These authors demonstrated that among age groups, the differences recorded were significant, and that coenurosis contributes to mortality, especially that of young animals.

According to Sharma and Chauhan (2006), due to the long period needed for cyst development, the onset of clinical signs normally occurs after 3 months of age. No significant differences were observed in terms of clinical symptoms among different species (Adane et al., 2015), but some studies report clinical differences between males and females (Deressa et al., 2012; Mirian et al., 2015). Not all animals positive for the presence of vesicles have clinical signs. A considerable proportion is diagnosed only after slaughter or necropsy (Sharma and Chauhan, 2006; Oryan et al., 2012). The absence of clinical signs in animals positive for coenurus may vary from 3% to 65% (Achenef et al., 1999).

1.1.3. Pathogeneses and clinical signs of *Taenia multiceps* larva

Coenurosis pathogenesis could be divided in two phases: the migratory and the growth phase. The early stages of migration through nervous tissue usually pass unnoticed, but in heavy infestation, meningoencephalitis may develop and it is difficult to diagnose. Most signs are caused by the mature cyst. Coenurus bladder cysts grow gradually and the increasing pressure on nervous tissue results in their inflammation and eventual necrosis (Dyson and Linklater, 1979; Soulsby, 1982; Radostits et al, 2000).

Subclinical coenurosis may be due to greater tolerance in some of the hosts, acquired immunity in older animals, recent infections or the age of the animal.

Clinical signs of cenurosis vary according to the location of the cysts. About 35 % of sheep exhibit disorientation, circular movements, stiff neck, loss of appetite, frequent bleating, unilateral partial blindness, head tilt to the right or left, incoordination, irregular march, ending with exhaustion and death. When located in the brain, *Coenurus cerebralis* is the cause of progressive fatal neurological manifestations and may exceed 30% mortality in acute cases (Kish et al., 2013). The cysts cause compression and may result in atrophy, necrosis in the surrounding tissues, hemorrhage, ataxia, neurological degeneration and meningoencephalitis (Kheirandish et al., 2012; Ioannidou et al., 2015). The presence of Coenurus cysts in skeletal muscles causes muscle pain, degenerative and necrotic changes and myositis (Chauhan and Sharma, 2006; Kheirandish et al., 2012). When observed in the heart, it causes granulomatous multifocal myocarditis (Kish et al., 2013). In the lungs, the tissue at the periphery of the vesicles reveals atelectasis focal interstitial fibrosis (Kheirandish et al., 2012). Ocular coenurosis, was diagnosed in Egypt, with the presence of a vesicle in the eyeball causing blepharitis and conjunctival congestion (Haridy et al., 2014).

Goats have greater diversity in the location of larval stages when compared to sheep, in which the location is primarily the brain (Achenef et al., 1999; Ozmen et al., 2005; Christodoulopoulos, 2007). In goats, the cerebral localization is not as frequent as in sheep (Nourani and Kheirabadi, 2009; Kheirandish et al., 2012).

In goats, non-cerebral sites may be subcutaneous tissues, abdominal cavity, diaphragm, pancreas, liver, kidney, heart, tunica adventitia of aortic lymph nodes, tongue, eye and muscles, most commonly intercostal muscles and thigh muscles (Sharm et al., 1995; Islam et al., 2006; Godara et al., 2011; Schuster et al., 2010; Oge et al., 2012; Kheirandish et al., 2012; Monsang et al., 2014).

Non-cerebral coenurosis was first observed in goats in India in 1907 by Gaiger, who considered it a new species.The parasite was taxonomically classified as *T. gaigeri* by Hall (1916) in honor of Gaiger (Varcasia et al., 2012). After the establishment of *T. gaigeri* as a species, the existence of two coenurus species was accepted. The one with extra-brain localization, ocurring mainly in goats, was designated *C. gaigeri*, while the one with cysts located in the central nervous system was identified as *C. cerebrali*s (Sharma et al., 1998; Oryan et al., 2010; Madhu et al., 2014; Monsang et al., 2014).

To further investigate the taxonomic position of these two species, morphological and genetic studies were conducted, comparing cysts collected from different animals and different anatomical locations.The results suggested that *T. multiceps* (*C. cerebralis*) was the only species and that the differences observed in the parasite's behavior were primarily due to differences in hostparasite interactions (Oryan et al., 2014; Varcasia et al., 2012; Kheirandish et al., 2012; Oge et al., 2012). Using experimental infections of definitive and intermediate hosts, followed by morphological and genetic analysis of adult and larval forms, Akbari et al. (2015) confirmed that *T. multiceps* (*C. cerebralis*) was a single species with different hosts and predilection sites.

1.1.4. Diagnosis of *Taenia multiceps* larva

The infection by the larval stage of *Taenia multiceps* is a zoonosis and is considered a neglected disease. Man could act as an intermediate host in the parasite life cycle, being infected in the same way as sheep and goats, by the ingestion of eggs passed by infected carnivores. The diagnosis of coenurosis in man is complex, requiring techniques and equipment, which are not always available (Wu et al., 2012). The larval stage (coenurus) may develop in the central nervous system, eye, subcutaneous and intramuscular tissue (Ing et al., 1998). The morbidity and mortality associated to brain coenurosis, particularly in African countries, have decreased mainly due to the increase of modern neuro-radiological techniques (Benifla et al. 2007). Ing et al. (1998) reported a case of cerebral coenurosis in a young man with extensive brain lesions. This case occurred in the United States of America, with full access to modern diagnostic facilities. Nevertheless, it took about seven months between symptom onset and the final diagnostic. Furthermore, a case of coenurosis in the eye, with late diagnosis, caused endophthalmitis, retinal damage and blindness (Ibechukwu and Onwukeme, 1991).

1.1.4.1. Direct diagnosis

The diagnosis of coenurosis is difficult because there are no pathognomonic signs and no reliable diagnostic tools. Physical and neurological examination together with general information on breed, age, sex, breeding condition, previous diseases, durations of signs and flock mortality are the basis to the diagnosis of cerebral coenurosis. A detailed protocol can be applied in order to localize the cyst in the brain which includes general behaviour, visual deficits, postural deficits, circling, head position and cerebellar signs (Skerrit, 1991;

Koumnenou et al., 2000). This protocol could be a useful clinical tool to employ on the diagnosis of cerebral coenurosis. However, it is essential to consider, the differential diagnosis, with other diseases. Listeriosis, cerebral cortical necrosis, louping-ill, visna, scrapie, brain abscesses (Radostits et al., 2000; Skerrit, 1991) and echinococcosis (Naghashyan and Hartunyan, 2001) have to be taken into the account in the differential diagnosis of *T. multiceps* larval infections. The definitive diagnosis for clinical coenurosis is only achieved by physical imaging methods like radiology (Tirgari et al., 1987), ultrasonography (Doherty et al., 1989) and computerized axial tomography (Gonzalo-Orden et al., 1999). These techniques are useful for the diagnosis of ovine cerebral coenurosis. They allow for overcoming the difficulties of interpretation of neurological sings and attaining an accurate localization of the cyst. However, mainly due to the cost of such technical procedures, in practice, they are seldom used. Post-mortem discovery of a thin-walled cyst filled with transparent fluid and with numerous scolices in the wall constitutes the definitive diagnosis (Soulsby, 1982; Kaufmann, 1996).

1.1.4.2. Indirect Diagnosis

1.1.4.2.1. Immunological diagnosis

Immunodiagnosis plays an important role in detecting early stages of illness. Assays such as; skin test for immediate hypersensitivity, indirect haemaglutination antibody (IHA) test, immunoelectrophoresis (IEP), gel double diffusion (DD), immunoblot and enzyme linked immunoassay (ELISA) test have been used experimentally in the diagnosis of coenurosis (Daud and Herbert, 1982; Skerrit and Stallbaumer, 1984; Price et al., 1989; Doganay et al., 1999).

The cyst fluid is the most common source of antigens used in the above-mentioned assays. . Metacestodes cyst fluid is a complex mixture composed of glico and lipoprotein, carbohydrates and salt driven from the parasite and host (mainly albumin and imonoglobulins). Crude fluid cyst (Price et al., 1989; Dognay et al., 1999), homogenate *T. multiceps* proglotides (Dyson and Linklater, 1979), fluid purified lipoprotein (Daud and Herbert, 1982) monoclonal antibodies from the onchospheres (TM 18) (Gaugi el al., 2008) and recently heat shock protein (TmHSP70) (Wang et al., 2015) have been used as antigen sources for the immunodiagnosis of *T. multiceps* larva.

Two studies evaluated the intradermal skin test for detection of cases with neurological disorders, but no differences were observed on skin reaction when compared with normal control lambs, using *T. multiceps* cyst fluid, *T. multiceps* proglottides and *T. hydatigena* cyst fluid as antigens (Dyson and Linklater, 1979; Skerrit and Stallbaumer, 1984).

In lambs with acute coenurosis, IHA test was used, but little correlation was obtained between test positivity and clinical pathology (Dyson and Linklater, 1979). Using a purified lipoprotein, IHA, IEP and DD tests presented higher sensitivity and specificity when compared with the non-purified cyst fluid on IHA (Daud and Herbert, 1982).

ELISA assay and immunoblot analysis were applied to diagnose *T. multiceps* larval stages in monkeys. Using coenurus cyst fluid, high titres of immunoglobulin G (IgG) and a response to seven protein bands (34, 37, 41, 88 KDa 3 bands >92.5 KDa) were obtained for ELISA and immunoblot respectively (Price et al., 1989). Furthermore, in an ELISA assay using crude cyst fluid on experimental infected sheep, seropositivity (antibodies) was detected from the 35th day post-infection (Dognay, et al., 1999). There are also reports on the use of a recombinant antigen, based on specific proteins from the oncosphere of *T. multiceps* (Tm16 and Tm18)in an ELISA to detect antibodies in sheep immunized with homologous proteins (Gauci et al., 2008). Recentely, Wang et al. (2015) evaluated an ELISA using the recombinant protein, TmHSP70 and reported high sensitivity and 83.3% specificity on a prelaminar evaluation with 30 *T. multiceps* infected goats.

Immunodiagnostic assays in other metacestodes such as porcine cysticercosis, neurocysticercosis (NCC), and echinococcosis (cystic (CE) and alveolar (AE)) are extensively used. The development of improved tools has contributed as an auxiliary diagnostic confirmation test, commonly applied in the management of disease treatment and for epidemiological studies (Dorny at al., 2003; Ortona et al., 2003; Zhang et al., 2007).

Antibody detection methods were developed based on the reaction of the humoural immune system to the infection with protoscolices. Infection with *T. solium* and *Echinococcus spp*. results in a specific antibody response, where the immunoglobulin G (IgG) class is predominant (Dorny et al., 2003; Ortona et al., 2003). Occurrence of antibody titres of other isotypes such as immunoglobulin M (IgM), immunoglobulin A (IgA) and immunoglobulin E (IgE) could also be present in smaller concentrations (Carpio et al., 1998; Zhang et al, 2007).

A great variety of serological tests have been developed for metacestode diagnosis in humans and animals. Tests with less sensitiviy and specificity including the Cassoni intradermal test, complement fixation test, the indirect haemaglutination test, and the latex agglutination test, have been replaced by the enzyme-linked immunosorbent assay (ELISA), dipstick ELISA, radioimmunoassay (RIA), the indirect immunoflurescence antibody test, immunoelectrophoresis (IEP) and immunobloting (IB) in routine laboratory applications (Lightowlers et al., 1996; Dorny et al., 2003). For these tests, the primary antigenic sources were the cyst fluid, crude homogenate of the parasite or related parasite such as *T. crassiceps* for *T. solium*. The major drawbacks of these crude antigens are their moderate sensitivities and relatively poor specificities (Fleury et al., 2001; Gracia et al., 2007). Due to these facts, purification techniques have been refined, resulting in much more reliable serological assays (Gosttstein et al 1986; Ito et al., 1999; Mayta et al., 2007; Gonzalez et al., 2007). For hydatid cyst fluid, two lipoproteins, antigen B (AgB) and antigen 5 (Ag5), were considered the most important diagnostic proteins (Oriol and Oriol, 1975).

The problems with sensitivity, specificity and the difficulties in standardization demonstrated in most of the antigens are mainly due to the high level of cross-reactivity with heterologous parasitic antigens. The use of recombinant proteins and synthetic peptides has the potential to circumvent the problem of cross reactivity improving the sensitivity and specificity of these diagnostic assays.

1.1.5. Molecular Identification of *Taenia multiceps*

Numerous species of Cestoda have been described as causative agents of diseases in humans and production losses in domestic livestock. Poor accessibility to specimens, incomplete taxonomic and morphological description and ontogenetic characters, are pointed as responsible for the current gaps in cestode systematics (Brooks et al., 1991, Mariaux, 1996). Notwithstanding, it is generally accepted that in the last 20 years, based on the combined research on morphology, life cycles, ultrastructural and molecular characteristics , the knowledge on the phylogeny of Eucestoda have improved substantially (Olson et al., 2005). Species identification/classification, relationship of species and epidemiological studies have been based on the extensive use of different molecular tools. Determination of phylogeny is based on features at DNA, protein, chromosomal and genomic level of organization. In the context of genetic characterization, it is essential to choose the appropriate technique, an adequate marker, and a robust analytical method (Thompson, 2004).

From the late seventies to nineties, studies on the molecular characterization of Taeniidae species, including *Taenia multiceps* were performed. Isoenzyme electrophoresis was the first assay used to differente the Taeniidae species (Le Richie and Sewell, 1978). The results of this study demonstrated intraspecific differences between *Echinococcus granulosus* (from horses), *Taenia hydatigena*, *T. multiceps*, *T. ovis*, *T. psiformis*, *T. saginata*, *T.solium* and *T. taeniaefomis*, using adenylate kinase, glucose phosphate isomerase and glutamate dehydrogenase. Later, a polymerase chain reaction-based restriction fragment length polymorphism technique (PCR-RFLP) was used to specifically identify seven species of Taenidae cestodes (*Echinococcus granulosus, E. multilocularis, Taenia hydatigena, T. ovis, T. pisisformis, T. multiceps and T. serialis*). The second internal transcribed spacer of ribosomal DNA (ITS2) was analysed and no variation in RFLP patterns was observed between *E. multilocularis* and other *Taenia* species. However, distinct intraspecific variation was detected for *E. granulosus*. The ITS2+PCR products of *T. multiceps* were similar to *E. granulosus*, *E. multilocularis* and *T. psisformis* (600- 620 bp) and different from *T. hydatigena* (550 bp) and *T. ovis* (540 bp). With this technique, it was possible to differentiate *T. multiceps* from *T. serialis* using the endonuclease AluI. These results highlighted the potential of PCR-RFLP of ITS2 for systematic, epidemiological and diagnosis purposes. (Grasser and Chilton, 1995). The sequences of the/a NADH dehydrogenase subunit and cythocrome c oxidase subunit 1 were used to identify a human case with coenurus (*Taenia serilais*) in France (Collomb et al., 2007). Furthermore, the same system was used to differentiate nine tapeworms of the genus *Taenia*. Gasser et al. (1999a) compare the sequence of the mitochondrial NADH dehydrogenase subunit 1 gene (~5.9 – 30.8%) and cytochrome *c* oxidase I (~2.5 – 18%) and demonstrate similarities on two groups specifically: i) *T. multiceps, T serialis, T. saginata* and the Asian Taenia, ii) *T. solium, T. ovis, T. hydatigena, T. pisiformis* and *T. taeniaeformis*. Figure 1 shows the phenograms based on ND1 and COI sequence data from the nine Taenia species studied. Intraspecific variability profiles for some taxa in eight Taenia species, including *T. multiceps,* was detected by single-strand conformation polymorphism (SSCP) technique in NADH dehydrogenase 1 and cytochrome *c* oxidase subunit I gene (Gasser et al., 1999b).

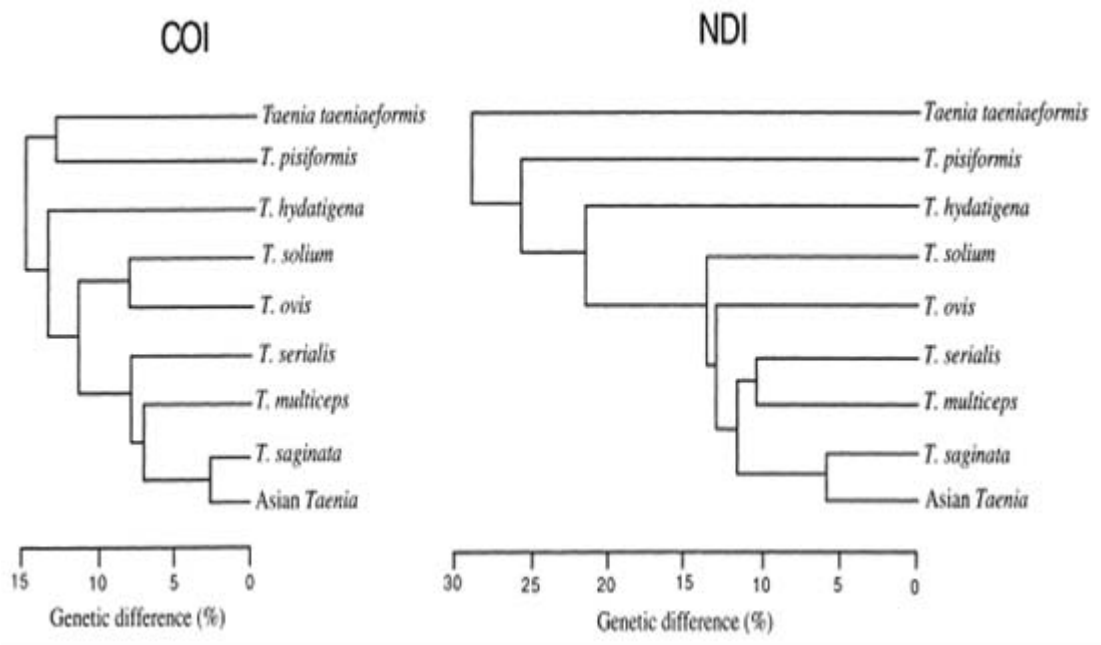

Figure 2 - Phenograms illustrate ng the genetic relationships among *Taenia taeniaeformis, T. hydatigena, T. pisisformis, T. ovis, T. multiceps, T. serilais, T. saginata, T. solium* and the Asian Taenia based on ND1 and COI sequence data

Source: Gasser et al., 1999a

Currently, molecular characterization of *T. multiceps,* is mainly applied to assess intraspecific genetic variability. In this context, in Sardinia, Italy, *T. multiceps* from sheep and dogs showed differences ranging from 1.27 to 2.54% and 0.22 to 0.67% for NDH dehydrogenase (ND1) and cytochrome c subunit 1 (COI) respectively, when compared to an isolate from Wales (AJ239104). Moreover, based on the same molecular markers, three genetic variants were defined and called Tm1 (AY669089), Tm2 (DQ309770) and Tm3 (DQ077820) (Varcasia et al., 2006). Similar studies conducted worldwide, in Italy (Varcasia et al., 2013), Iran (Oryan et al., 2010; Rostami et al., 2013; Akbari et al., 2015; Amarabadi et al., 2015), Turkey (Avciogulo et al., 2011), United Arabs Emirates (Varcasia et al., 2012) and China (Li et al., 2013; Li et al., 2015) demonstrated the existence of different strains. *Taenia multiceps* genetic variability could be related to differences in its epidemiology and clinical forms observed in *Echinococcus spp*., which belongs to the same family (Bowles and McManus 1992; McManus 2005). As there are no reports on molecular characterization of *T. multiceps* from Africa, this study was carried out to provide insights into the identification of CO I (cytochrome c subunit 1) and ND1 (NADH dehydrogenase 1) mithochondrial gene sequences of *T. multiceps* from goats in Mozambique.

1.1.6. Treatment and control of *Taenia multiceps* larva

Surgical removal of cysts and the application of anthelmintics are the two methods reported for the treatment of coenurosis. The most effective treatment to cure gid is the surgical removal cysts. The accurate localization of the cyst in the brain is an essential prerequisite to apply this technique. Success rate of this treatment can be as high as 74 % (Skerrit and Stallbaumer, 1984; Tirgari et al., 1987; Komneou et al., 2000). But there are a proportion of infected animals were it is impossible to locate and remove the cyst. After successful removal of the cyst the condition of some animals (36/623) did not improved or deteriorated and others (56/623), after the surgical procedure, showed a temporary improvement, but later their condition deteriorated and they had to be slaughtered (Komnenou et al., 2000).

As alternative to surgical removal therapy with praziquantel, fenbendazole and albendazole have been tested to treat coenurosis in sheep. Praziquantel has been used in treatment of acute and early infection of *T. multiceps* larva. Different doses, from 25 to 100 mg/kg, where used during a period of 5 to 14 days (Table 1). The efficacy of the treatment was good but it was very expensive and not affordable for the majority of production animals.

Table 1 - Different treatments for *Taenia multiceps* larva with Praziquantel

Animals **Course of infection**	**Doses** **Duration of treatment**	**Efficacy**	**References**
Sheep Acute	100 mg/Kg 5 days	8/9 dead cyst 1/9 immature	(Verster and Tustin, 1982)
Sheep Infection and treatment	25 mg/Kg 6 times intervals 15-20 days	No cyst development (good chemoprophylaxis)	(Aminzhanov et al. 1988)
Lambs Acute	25 mg/Kg 14 days	100% (35/35) degenerated or dead cyst	(Biyikoglu and Doganay, 1998)
Sheep Acute	100 mg/Kg 7 days	Removed clinical signs and cyst (4/5)	(Ghazaei, 2007)

Fenbendazole was tested against *T. multiceps* larva, Aminzhanov *et al*. (1988) administered ten days after infection at a dose of 0.7-1 g per animal six times during an interval of 15-20 days. With this treatment no cyst development were observed at necropsy. Ghazaei (2007) tested two treatments, in an acute phase of coenurosis, fendendazole at doses of 25 mg/kg during 8 days, and 3 ou of 5 sheep were successfully treated. In another experiment, using natural infected sheep with acute coenurosis (n=5), were treated with an association of praziquantel (100 mg/kg) and febendazole (0.5g/animal) and cure was observed in all animals.

Albendazole is commonly used for the treatment of various metacestodes. Two trials were done in order to assess the efficacy of albendazole against *T. multiceps* in sheep. Both studies used the drug for a period of 6 to 14 days and the observed efficacy of 54.7 and 100% in the acute form of the disease, details are shown in table 2.

Table 2 - Treatment of *Taenia multiceps* with Albendazole

Animals **Course of infection**	**Doses** **Duration of treatment**	**Eficacy**	**References**
Lamb Acute	100 mg/Kg 14 days	54.7% (23/42) Degenereted or dead cyst	(Biyikoglu and Doganay, 1998)
Sheep Acute	25 mg/Kg 6 days	Removed clinical sings (5/5)	(Ghazaei, 2007)

Coenurosis control is a complex process.The parasite life cycle involves several species of carnivores that can play a role of definitive host and a large number of herbivores and omnivores that could be intermediate hosts. Complex biological interactions between the parasite and host, including different strategies to avoid the host immune system, are aspects that interfere with the effectiveness of control. Some vaccines trials with antigens from *T. multiceps* oncospheres showed significant reduction of animals infected with *C. cerebralis* (Gauci et al., 2008; Varcasia et al., 2009; Scott, 2012).

In developing countries where coenurosis is an important problem for animal production, the adoption of vaccination as a control method is difficult and not feasible due to economic limitations of the population and the logistic of vaccines distribution.

Factors such as the close contact between dogs and small ruminants (sheep and goats), lack of knowledge of the population about how coenrusis occurs, free access of dogs to the viscera of ruminants or rejected parts, containing coenurus vesicles, the absence regular deworming of dogsare the most important drivers for prpetuation of *T. multiceps* cycle and the persistence of coenurosis (Gicik et al., 2007; Tavassoli et al 2011; Miran, 2015).

Treatment of dogs as well as the appropriate treatment of the viscera and carcasses after slaughter, are considered to be fundamental in the control of coenurosis (Sharma and Chauhan, 2006; Wu et al., 2012).

So, a sound control strategy requires control methods that not only demonstrate efficacy, but more importantly are affordable and easy to implement on the ground. Population education

and dog treatment with effective anthelmintics are the most effective way to break the cycle of the parasite and thus counteract the persistence of this disease.

1.2. Albendazole

Albendazole (ABZ) belongs to the benzimidazole (BZD) anthelmintic drug group. Thiabendazole was the first anthelmintic drug to be introduced in 1961 (Horton, 1990). In the first decade after its introduction, subsequent modifications were made to its molecular structure to generate safer and more efficacy drugs. Within the BZD compounds utilized as anthelmintic, four main groups could be identified, namely: i) thiazolyls ii) methyl carbamates iii) halogenated and iv) pro-benzimidazoles.

Albendazole, a broad spectrum anthelmintic, has been used for the treatment of human and animal diseases. BZD is well tolerated by mammals and its spectrum of activity involved a variety of adult and larva of helminths located in gastrointestinal tract, lungs and blood (Mckeller and Scott, 1990). This drug is a compound white crystalline powder soluble in dimethilsulphoxid, strong acids, strong bases and alcohols. Chemical structure of ABZ is (5-(propylthio)-1H-benzimidazol-2-etyl) (Brander et al., 1977) (**Figure 3**). Chemical formula of ABZ is $C_{12}H_{15}N_3O_2S$ and its molecular weight is 265.333 g/mol.

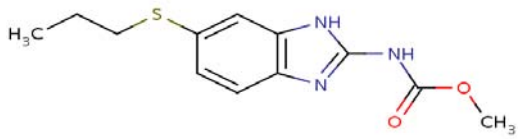

Figure 3 - Chemical structure of albendazole

The formulation of ABZ is dependent of its poor water solubility. As a result, this drug could be found as suspensions or pellets for oral/intraruminal administration (Lanusse and Prichard, 1993; McKellar and Scott, 1990). However, a parenteral formulation of albendazole suplhoxide (ricobendazole) has been developed by exploiting its greater hydrosolubility compared to other BZD molecules (Lanusse et al., 1998).

1.2.1. Pharmacokinetics of Albendazole

1.2.1.1. Absorption and distribution

Factors related to anatomical and physiological parameters of animal species, as well as physico-chemical proprieties of the drug such as mucus surface and degree of ionization at different pH values, determine the absorption rate. At normal rumen pH (6.5-7.0), solubility of BZD compounds is low but it increases at the lower pH (2-3) encountered in the abomasums and upper small intestine (Marriner et al., 1985). After oral and ruminal BZD administration, absorption takes place in the gastrointestinal tract, occurring faster in monogastric animals than in ruminants. Benzimidazole persists for longer periods in ruminant forestomachs where it is absorbed and consequently stays in plasma for a longer-lasting effect. Absorption is also influenced by the oesophageal groove closure which occurs in nursing ruminants. This reflex reduces plasma bioavailability by the complex absorption process which takes place when a drug is administrated orally and this solution divided between the ruminorecticulum and abomasum (Prichard et al., 1985; Hennessy et al., 1989). Nevertheless, when the drug is administered intraruminally, ABZ is absorbed unchanged from the rumen.

The rate of absorption and recycling, between enteral and parenteral tissues, are relevant and depend on the exposure of worms residing in the lining of the gut to the active drug and its metabolites. Furthermore, it was shown that the absorbed drug is more important for the efficacy against gastrointestinal nematodes than an unabsorbed drug passing down the gastrointestinal tract (Hennesy and Prichard, 1981). Consequently, the plasma concentration profiles of antihelmintic active BZD moiety reflect the pattern of exposure of worms in the gastrointestinal tract (mucosa or lumen) as well as other tissues of parasite location (Cristòfol et al., 1997a; Alvarez et al., 1999; Cristòfol et al., 2001).

To overcome problems related to bioavailability limitation, several strategies have been studied in animals and humans such as formulation additives and modification of condition at gastrointestinal tract. In animals, formulations/additives with ABZ were developed by the co-administration of an oily water soluble liquid, diethyl glycol monoethyl ether (Torrado et al.,

1997), surfactants/liposomes (Wen et al., 1996), and solid dispersions (Torrado et al., 1997; Kohri et al., 1999), by inclusion complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD) (Piel et al., 1999; Evrard et al., 2002; Gracia et al., 2003), and by addition of a surfactant agent (Virkel et al., 2003) or as monoparticles (Rodrigues et al., 1995). In humans, properties of three formulations; guar gum-based colon targeted matrix tablets of albendazole-β-cyclodextrin (KA), albendazole matrix tablet (GA) and an immediate-release albendazole tablet (CA), were assessed (Shyale et al., 2006). From this clinical trial, it was possible to observe that KA and GA tablets presented delayed Tmax and absorption time, decreased absorption rate constant and unaltered t1/2, indicating that albendazole was delivered to the colon consequently making the drug available for the local action in the colon.

Condition of the gastrointestinal tract - Diet and other factors affecting the ruminal development such as physical and chemical interaction, dilution effects and pH modifications in the rumen, have been shown to affect the rate of absorption of benzimidazole drugs. The acidic abomasal pH facilitates the dissolution and the absorption in the lower gastrointestinal tract (Hennessy, 1993; Lanusse and Prichard, 1993). In ruminants, absorption can be significantly improved when benzimidazole is administrated to the empty rumen (Lanusse and Prichard, 1993; Sanchez et al., 1997). Moreover, an increase of concentrations of ABZ metabolites was observed in plasma resulting in a high peak drug plasma concentration (Cmax) and area under the curve (AUC) values in concentrate-fed calves (Sanchez et al., 1999) and in sheep with dry food (Singh et al., 1999). It has been reported in humans that bioavailability of albendazole was improved with a fatty meal (Lange et al., 1988), with grapefruit juice (Nagy et al., 2002) and with soybean oil emulsion (Mingjie et al., 2002).

ABZ is absorbed in the gastrointestinal tract and is rapidly converted into its metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphona ($ABZSO_2$) (Mariner and Bogan, 1980; Hennessy et al., 1989; Lanusse et al., 1995). Distribution of ABZ metabolites throughout the body occurs via the circulatory system. *In vitro* and *in vivo* studies were performed in order to estimate the amount that ABZ metabolites bind to plasma protein. During an *in vitro* study it was demonstrated that ABZ metabolites bind to plasma protein from 50.5 to 87.8%, and from 84.5 to 92.6% for ABZSO and $ABZSO_2$ respectively (Cristòfol et al., 1998). Throughout an *in vivo* study in sheep, the results for ABZ were 42% at 12 h, 48% at 24h, 87% at 36 h and 100% thereafter (Hennessy et al., 1989). When comparing the

plasma protein binding metabolite profiles, $ABZSO_2$ is more extensively bound than ABZSO.

For ABZSO disposition in ruminants it is important to considerer the effect of ionic trapping which occurs in the abomasum (Hennessy, 1993). This event is demonstrated by the fact that after 30-36 hours post treatment plasma concentration of ABZ metabolites fell to undetectable levels but profiles of these metabolites in the rumen, abomasum and ileum showed the extent on elimination phase. This metabolite-distribution process may be driven by a plasma/gastrointestinal tract pH gradient and pK_a of the drug (Lanusse and Prichard, 1993). In figure 4 the schematic pathway of BZD disposition on the gastrointestinal tract in ruminants is represented.

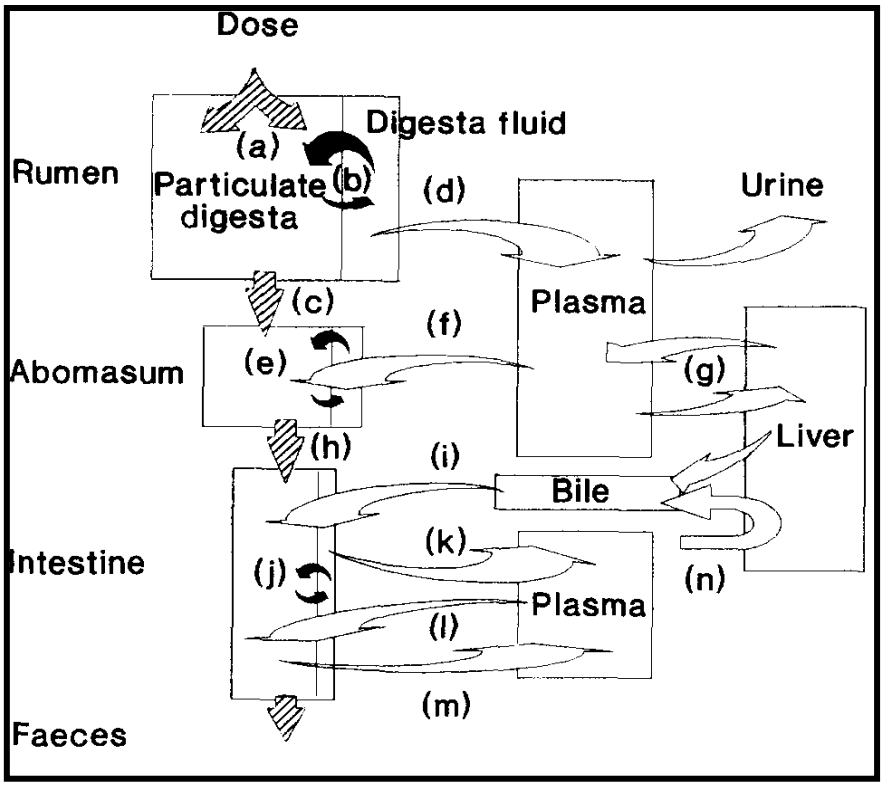

Figure 4 - Schematic representation of benzimidazole coumpounds on ruminant gastrointestinal tract (source: Hennessy, 1993)

a) BZD drug in rumen are mixed with digesta particulate b) and digesta fluid an equilibrium of drug (parenteral and metabolites) between the particulate and fluid c) a proportion of drug parenteral/metabolites flowed from rumen to abomasum d) and other proportion of the drug parenteral/metabolites were absorbed from digesta fluid to bloodstream e) drug in abomasum remains predominantly associated with particules material f) drug from bloodstream to the abomasum (ionic trapping) g) following the absorption the drug is oxidized in liver h) a fraction of drug flow from the abomasum to the intestine i) bile secreted metabolites re-entreded on intestine j) on intestine a proportion of

biliary metabolites are re-absorbed and enterohepaticlly recycled k) on upper small intestine re-absorption of metabolites l) intestinal secretion, passive metabolites transfer from plasma to intestine m) metabolites reabsorbed from large intestine n) metabolites transport via portal circulation and re-secreted in bile

ABZ metabolites appear to have a good distribution for all tissues. This statement was been confirmed by findings after intravenous administration of ABZ to sheep (Galtier et al., 1991) and ricobendazole to calves (Cristòfol et al., 2001) showed that the disposition curves had an initial rapid distribution phase followed by a longer elimination phase. In humans, it was shown that ABZSO has the ability to cross the blood-brain barrier. This ability was demonstrated by its efficacy against neurocysticercosis (Sotelo and Jung, 1998). The transport of ABZSO is passive by drug diffusion of the blood-brain barrier trough (Takayanagui et al., 2002).

1.2.1.2. Metabolisme

Principles involving biotransformation reactions for BZD were possible to recognize from the metabolites found in plasma, urine, and other tissues and from *in vitro* studies. The major effect on the ABZ metabolism is the placement of a sulphur atom in the BZD side chain which is characterized by the oxidation of the nucleophilic heteroatom (Gottschall et al., 1990). Albendazole biotransformation has been studied in different species including rat (Fargetton et al., 1986; Delatour et al., 1991b), mouse (Douch and Buchanan, 1979), rabbit (Li te al., 1995), poultry (Li et al., 1995), sheep (Marriner and Bogan, 1980), goat (Delatour et al., 1991a), cattle (Prichard et al., 1985), pig (Souhaili-El Amiri et al., 1987), dog (Delatour et al., 1991b) and man (Penicaut et al., 1983). After the administration of ABZ, the parent compound is undetectable in plasma (Villaverde et al., 1995).

It is known that this sulphide-type of anthelmintic undergoes a two-step S-oxidation giving firstly albendazole sulphoxide (ABZSO) followed by albendazole sulphone ($ABZSO_2$). ABZSO has two antipodes, (-)-ABZSO and (+)-ABZSO produced from the chiral thioether albendazole, which can be separated by HPLC; (+)ABZSO is associated with the activity of flavine monoxygenase (FMO), whereas the P-450 system participates in the production of (-)-ABZSO (Delatour et al., 1991b). Metabolic pathway of albendazole is represented in Figure 5.

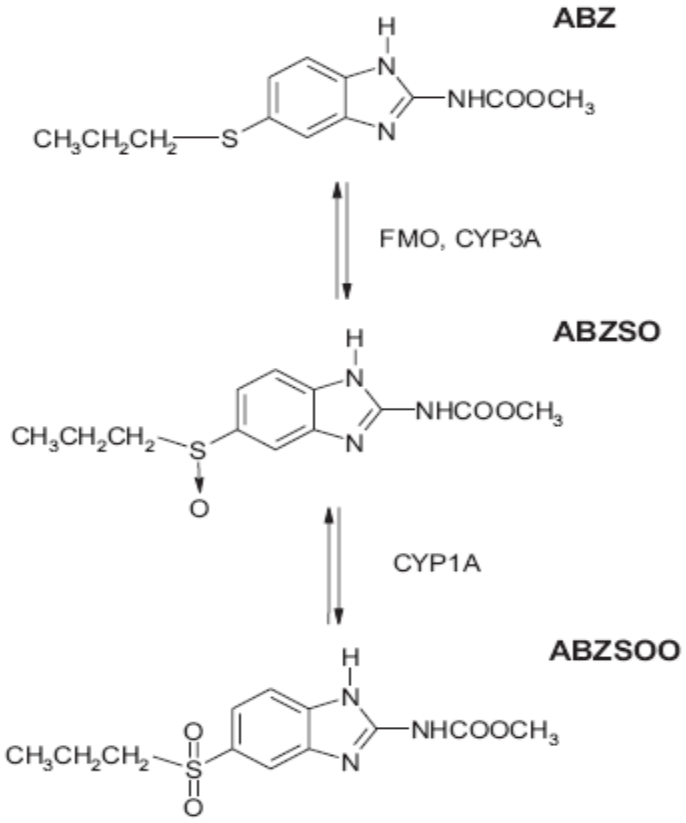

Figure 5 - Metabolic pathway of albendazole

Gastrointestinal metabolism - It was demonstrated that the liver microsomal fraction is considered to be the principal site for biotransformation of BZD compounds but the gastrointestinal metabolism also takes an important role in pharmacokinetic behaviour and the availability of anthelmintically active metabolites at the sites where gastrointestinal and tissue dwelling parasites are located. Gastrointestinal fluids and enterocytes are responsible for the BZD metabolism in the gastrointestinal tract (Lanusse and Prichard, 1993). Due to the presence of specific microflora and physiochemical conditions, a gastrointestinal tract reaction takes place involving oxidation, reduction and hydrolisis (Lanusse and Prichard, 1993). Effect of ruminal microflora on biotransformation of BZD compounds had been reported in *in vitro* studies by Lanusse et al., (1992a, 1992b), Capece et al., (2001) and Virkel et al., (2004). It has been suggested that ruminal metabolism is not enantioselective since racaemic equilibrium is observed following ruminal fermentation using artificial rumen model (Capece et al., 2001).

Oxidation of ABZ to ABZSO occurs at intestinal level with the participation of flavin-containg monoosygenase (FMO) (Fargetton et al., 1986, Galtier et al., 1986, Lanusse et al.,

1993, Moroni et al., 1995, Villaverde et al., 1995) and cytochrome P-450 (CYP) (Souhaili-El Amiri et al., 1987) enzymatic systems. Previous studies on liver and intestine microsomes of rat have demonstrated that both systems are similarly involved (Moroni et al., 1995, Villaverde et al., 1995, Redondo et al., 1999). It was observed *in vitro*, in intestinal microsomal from rats that ABZ was captured by enterocytes and then metabolized into ABZSO (Villaverde et al., 1995).

Hepatic metabolism - The main albendazole biotrasformation occurs in the liver. Successive oxidations lead to active metabolites albendazole sulfoxide (ABZSO) in a reversible process, and albendazole sulfone ($ABZSO_2$) which has less anthelmintic activity (Lacey, 1990, Lanusse and Prichard, 1993). Sulfoxide and sulfone metabolites dominate the plasma profile and are also found as the major metabolites in urine, which is the main route of albendazole metabolites elimination, although the exact proportion of each varies considerably among species (Gottschall et al., 1990).

Liver microsomal fraction: the flavine monoxygenase (FMO) (Fargetton et al., 1986, Morini et al., 1995) and cytochrome P-450 (CYP) (Souhaili-El Amri et al., 1988b) enzymatic systems are responsible for the biotransformation of ABZ. FMO and CYP3A participate in the first metabolic step, from ABZ to ABZSO whereas the formation of the sulfone is mainly catalyzed by CYP1A subfamily (Souhaili-El Amri et al., 1988a). These findings are supported by the fact that after administration of ABZ in sheep and cattle the peak plasma concentration of $ABZSO_2$ metabolite was delayed compared with that of ABZSO (Marriner and Bogan, 1980, Hennessy et al., 1989). ABZ has been shown to modulate activity and has the potency to induce CYPs in rat and human (Souhaili-El Amri et al., 1988a, Rolin et al., 1989, Asteinza et al., 2000). Induction has been observed in goats when successive doses of ABZ were applied to the amount of $ABZSO_2$ increases and decrease the ABZSO (Delatour et al., 1991a, Benoit, 1992). However, in an *in vitro* study with hepatic microssomes from rat and mouflon, ABZ and ABZSO demonstrated a significant potency to inhibit CYP activities (Baliharová et al., 2005).

Following hepatic metabolism, the active metabolite and/or the parent drug undergoes prolonged recycling between the enteral and parenteral tissues. This recycling has a great influence on the duration of their systemic availability.

1.2.1.3. Excretion

ABZ is mainly eliminated via urine and in a minor proportion by faecal excretion (Hennessy et al., 1989, Capece et al., 2008). In sheep, after an intraruminal dose of ABZ, it was observed that a large proportion (70 – 80%) of the albendazole dose was excreted rapidly in urine and a small proportion (14.3%) by biliary secretion. From the product secreted in bile 56% was present as unconjugated (8% dose) and 44% as conjugated metabolites (6.3% of dose). The major conjugated biliary ABZ metabolites were 2OH-ABZSO and 2OH-$ABZSO_2$ (Henessy et al., 1989).

Following an administration of ABZ to donkeys it was demonstrated that faecal excretion occurred and the ratios of faecal AUC of ABZ:ABZSO were 1:1 (Gokulut et al., 2006b). The authors suggested that high intestinal concentration of ABZ and ABZSO were due to intestinal metabolism. In ewes, after oral administration of netobimin (NTB), the parental drug was not detected at faecal excretion but the AUC of ABZ was higher than its metabolites (ABZSO and $ABZSO_2$) (Gokulut et al., 2006a). Fast reduction of NTB to ABZ was detected in artificial rumen and ruminal and intestinal fluids. This pharmacological behaviour could lead to an increment in the effectiveness against gastrointestinal parasites which live in gut lumen and the ineffectiveness against migrating larvae. In rats it was observed that the elimination of ABZ metabolites could be increased with the co-administrations of ginseng (*Panax ginseng*) (Merino et al., 2003a).

1.2.3. Factors affecting pharmacological parameters

Pharmacological parameters are influenced by a number of host-related factors. Studies on BZD compounds indicated that nutritional status, feeding management and disease could influence the amount of active BZD drug which reaches the parasites and the length of time that parasites are exposed to drug concentration. Animal species, age, sex, gestation, food intake, presence of gastrointestinal parasites or diseases may have an important influence on the bioavailability and efficacy.

There are marked differences in the pharmacokinetic profiles of BZD anthelmintics between animal species. In dogs, Gokbulut et al.,(2007) observed that with higher doses of ABZ and fedendazole, pharmacokinetics parameters were lower and shorter than the parameters in ruminants. This fact was justified by the faster passage through the gastrointestinal tract when

compared with ruminants. Differences have also been reported between ruminants. The half-life and mean residence time of ABZSO and $ABZSO_2$ after ABZ or netobimin administration were higher in sheep than in cattle. Additionally, plasma ratio of $ABZSO_2$/ABZSO was higher in cattle than in sheep (Gyruik et al, 1981; Delatour er al., 1990; Lanusse et al., 1992b; Lanusse and Prichard 1993). When pharmacological parameters were compared in small ruminants after administration of ABZ, sheep tended to present plasma disposition profiles and pharmacological parameters of ABZSO and $ABZSO_2$ higher than goats (Delatour er al., 1991a; Benoit, 1992; Sangster et al., 1992; Benchaoui et al., 1993; Hennessy et al., 1993b; Sanyal 1998a, 1998b). Hennessy et al., (1993b) suggest that there is a need for higher ABZ doses in goats than in sheep.

Several studies, in animals and humans, have described differences related to gender on the disposition of drugs in the body. In humans, many factors contribute to interindividual pharmacokinetic variability, in general related to hormone production, for example: physiology of intestinal tract, hepatic enzyme production (cytochrome P450 system) and corporal body mass. Variations, between gender were observed for some pharmacokinetic parameters of ABZ metabolites in human (Mirfazaelian et al., 2002) and sheep (Cristòfol et al., 1998). These differences were suggested to be associated with sexual hormones, plasmatic proteins, body water and enzymatic production in males and females (Cristòfol et al., 1998; Capece et al., 2000). It has been demonstrated, in an *in vitro* study, that plasma protein binding of ABZ metabolites was lower in males than in females (Cristòfol et al., 1998). Nevertheless, no differences were observed in pharmacokinetic parameters of ABZ metabolites in goats (Capece et al., 2009).

The gastrointestinal tract in young ruminants behaves as in monogastric animals. Until the rumen becomes fully functional, at 8-12 weeks, the esophageal groove may also act to bypass the rumen which normally slows the absorption of insoluble drugs such as the BZD (Hennessy et al., 1989). It is probable that even when the reflex occurs, the drug remains spread along the esophageal groove and omasum and will show a biphasic pattern of absorption (Bogan and Marriner, 1985). Differences were observed in pharmacokinetic parameters of ABZ metabolites in young and adult goats (Capece et al., 2009). Nevertheless, no significant differences in plasma disposition of metabolites were observed with age of the lambs after oral administration of netobimin and ABZ and ABZSO (McKeller at al., 1993, 1995).

Pharmacokinetic and plasma levels of metabolites were similar in pregnant and non pregnant rats after oral administration of NTB indicating that the elimination pattern of ABZ and its metabolites is not affected by the early stage (10 days) of pregnancy (Cristòfol et al., 1997b).

Fasting induces delay in gastrointestinal transit time and substantially increases the plasma and abomasal availability of ABZ in cattle (Sánchez et al., 1997; 2000) and sheep (Lifschitz et al., 1997; Singh et al., 1999). Several reports, using differing diets and animal breed/age, demonstrate the impact of feeding management on plasma concentration versus time profiles of different anthelmintic compounds in sheep (Ali and Chick, 1992; Taylor et al., 1993; Oukessou and Chokounda, 1997; Singh et al., 1999), cattle (Sánchez et al., 1999) and pigs (Alvarez et al., 1996). Moreover, different types of diets have been shown to affect ruminal pH and modify microflora-mediated metabolic sulphoreduction of BZD derivates (Virkel et al., 1997), additional influencing the disposition kinetics of these compounds in ruminants.

Cmax and AUC of ABZSO and $ABZSO_2$ were higher in pigs grazing on pasture than animals fed on concentrate and whey with corn grain (Alvarez et al., 1996). On the other hand, higher Cmax and AUC values for ABZSO and $ABZSO_2$ were found in calves fed on concentrate diet than the others fed on pasture (Sánchez et al., 1999). In sheep, peak plasma concentrations and availability of ABZ metabolites were higher in animals fed exclusively dry folder compared to other diets. These results suggested that a decreased transit time of the digesta in the bowel in the green diet limits the systemic availability of the drug by reducing the time available for gastrointestinal absorption (Singh et al., 1999). Similar results were observed in lambs with administration of triclabendazole, lower availability was shown in grazing animals compared with housed lambs (Taylor et al., 1993).

The presence of parasites could induce important changes to pharmacokinetic behaviour, side-effects and expected efficacy of the chosen anthelmintic for therapy.

Inflammatory reactions caused by parasites lead to alterations in mucosal permeability and in abomasal/intestinal pH, and this could have an impact on absorption and distribution of different BZD metabolites. During abomasal parasitism there is an elevation in abomasal pH and increased permeability of the mucosa to macromolecules (Sykes, 1978; Dakkak, 1984). Gastrointestinal parasitism due to the pathophysiological changes may affect absorption of different drugs due to modifications in the gastrointestinal transit time and atrophy of intestinal villi (Sykes, 1978). Influence of gastrointestinal parasitism on plasma kinetics of

anthelmintic drugs differs according to nematode species and degree of infection and anthelmintic compound involved. A study with lambs infected with a low burden of *Nematodirus battus* (intestinal nematode) showed no marked kinetic changes in ivermectin, levamisol or netobimin (Mckeller et al., 1991). However, following an artificial infection with abomasal nematodes (*Ostretagia circumcita* and *Haemonchus contortus*) the pattern of absorption and systemic availability of febendazole (Marriner et al., 1985), oxfendazole (Hennessy et al., 1993a), febantel (Debackere et al., 1993) and albendazole (Alvarez et al., 1997) in sheep have been shown to be altered. Peak plasma concentrations for ABZSO and $ABZSO_2$ were higher in both artificially and naturally infected sheep than in non parasitized animals with *Haemonchus contortus* (Alvarez et al., 1997).

Liver parasites can also modify the host's ability to metabolize drugs by altering the biotransformation enzymes. BZD metabolism occurs predominantly in the liver, thus, those parasites that occupy sites in this organ (such as *Fasciola spp*. or *Dicrocelium spp*.) tend to be the ones with the greatest effects on the host's ability to metabolize drugs. It has been demonstrated, in sheep infected with *F. hepatica*, that sulphonization of ABZSO were reduced and caused a significant decrease of activity level for CP450, but the FMO activity remained unchanged (Galtier, 1991). In studies with sheep infected with *F. hepatica*, a decrease of CYP3A expression and activity in hepatic microssomes was shown (Jemili et al., 1994; Calléja et al., 200). However, a significant increase in enzyme related to FMO and a significant decrease of glutathione-*S*-transferase (GST) activity was observed in mouflon (*Ovis musimon*) with *Dicrocelium dendriticum* infection after administration of ABZ (Skálová et al., 2007). An inductive effect on P4501A was observed in mouflon (*O. musimon*) parasited with *Dicrocelium dendriticum*, after the administration of a single dose of ABZ (Lamka et al., 2007). In rats, infected with *F. hepatica*, a decrease of activities drug metabolizing enzyme and an increase of activities CYP3A and CYP1A was observed (Biro-Sauveur et al., 1995).

1.2.4. Mode of action

All compounds belonging to the benzmidazole group are thought to have a similar mode of action and differences in their efficacy seen to be caused by differences in their bioavailability (Barragry, 1994). Benzimidazole has a broad spectrum of activity against a variety of pathogenic internal parasites. In susceptible parasites, their mechanism of action is believed to be due to disrupting intracellular microtubular transport systems by binding

selectively and damaging tubulin, preventing tubulin polymerization, and inhibiting microtubule formation. Benzimidazoles also act at higher concentrations to disrupt metabolic pathways within the helminth, and inhibit metabolic enzymes, including malate dehydrogenase and fumarate reductase.

The activity is based on the inhibition of energetic metabolism and inhibition of tubulin polymerization of microtubules from the parasite (Lacey, 1990 and McKellar; Scott, 1990). This section describes the two modes of action of the BZD compounds.

Inhibition of tubulin polymerization of microtubules - Microtubules are intracellular organelles that serve a variety of functions such as; movement of chromosomes during cell division, providing the structural skeleton to the cell, movement of intracellular particle including energy metabolites and exocytosis. They are found in animal, plant, fungi and some bacterial cells (Martin, 1997). Microtubules are composed of dimeric proteins known as α-tubulin and β-tubulin of approximately 50 KDa each (Lacey, 1990). The formation of microtubules is a dynamic equilibrium process, involving polymerization of tubulin at one end (the positive pole) and depolymerization at the other end (the negative pole) being controlled by a range of endogenous regulatory proteins and co-factors (Lacey, 1990; Martin, 1997). Exogenous substances known as microtubuline inhibitors could induce changes in this equilibrium. Most of such inhibitors apply their action by binding to tubulin to prevent the self-association of subunits onto growing microtubules (Lacey, 1990). Thus, the mode of action of the BZD anthelmintics is the selective binding to parasite β-tubulin following an inhibition of microtubule formation causing a mitotic inhibition (Lacey, 1990; Martin, 1997; Oxberry et al., 2001). As a result of the inhibition of microtubeline polymerization a disappearance of cytoplasmic microtubules causes disruption in the migration of subcellular organelles and a failure in the transport of secretory granules which leads to the blocking of several vital processes in the parasite, such as motility and nutrient uptake (McKellar and Scott, 1990). In an *in vitro* study with *Trichinella spiralis* it was demonstrated that several BZD (mebendazole, febendazole, oxibendazole and albendazole) inhibited in a competitive manner colchicine binding indicating an affinity for this biding site (Jiménez-González et al., 1991). Point mutations in β-tubulin subunits have been implicated in BZD resistance strains that are located at the periphery of the putative benzimidazole-binding site (Robinson et al., 2004). Recently, it has been shown that mutation in glutamate-198 to alanine correlates with thiabendazole resistance in *Haemonchus contrortus* from South Africa and Australia (Ghisis

et al., 2007). These authors suggested that this mutation plays a role in nematode BZD resistance.

Inhibition of energetic metabolism - Among prophylactic anthelmintics, there are some that affect the energy metabolism of parasites alone, possibly due to variations in the metabolism of the parasite and the host. They act by inhibition of enzymes related to respiratory metabolism pathways including phospoenolpyruvate carboxykinase, malate dehydrogenase (LDH), and the furamate reductase, succinoxidase decarboxylase systems (Behm and Bryant, 1979). BZD compounds disturb the energy metabolism of parasites. Effects of ABZ on lactate dehydrogenase (LDH) activity of *Cotylophoron cotylophoron* were studied in vitro and it was observed that significant inhibited activity of LDH catalyzed the oxidation of lactate while accelerated activity of LDH catalyzed the reduction of pyruvate (Veerakumaru and Munuswamy, 2000).

1.2.5. Efficacy

The anthelmintic activity of BZD compounds depends on their affinity for a specific receptor (β-tubulin) and the transport properties that allow the delivery of effective concentration of the compound at the receptor within the parasite cells, in sufficient time to cause the therapeutic effect (Thompson et al., 1993). Albendazole is a broad spectrum anthelmintic active against adult and immature stages of nematodes, cestodes and trematodes. In veterinary medicine it is used against gastrointestinal nematodes, lungworms, tapeworms and in higher doses against liver fluke (Campbell, 1990). In human medicine, it is recommended for the treatment of gastrointestinal parasitic infection, microfilaria, hydatid disease and neurocysticercosis (NCC) (EMEA, 1997; Dayan, 2003).

1.2.5.1. Efficacy in animals

ABZ is approved for the treatment and control of nematodes in several animal species. In ruminants, several regimes have been used to treat and control gastrointestinal nematodes with high efficacy (Onar, 1990; Chatier et al., 1996; Williams et al, 1997; Moreno et al., 2004; Rruvalcaba et al., 2006). ABZ is one of the choice drugs to use in lungworm infection in ruminants as strategic anthelmintic treatments which include both prophylactic treatment and treatment of carrier (Helle, 1986; Osman et al., 1996; Panuska et al., 2006). Recommended doses for lungworms are 7.5 and 10 mg/kg (Panuska et al., 2006) or at sub-

lethal doses of 2 mg/kg for 10 days (Osman et al., 1996) for cattle, and 1.25, 2.5 and 5 mg/kg in food for one or two weeks for goats (Helle, 1986). In studies conducted in sheep, ABZ promoted non-viable hydatic cysts of *Echinococcus granulosus* (Santos et al., 2008) and removed all signs in acute coenorosis caused *T. multiceps* larva (Ghazei, 2007). However, in experimental acute coenurosis in sheep, ABZ was only fairly effective (54.7%) (Biyikoglu and Doganay, 1998). A dose dependent study carried out with experimental infected goats with *Fasciola hepatica* demonstrated that ABZ had more efficacy at 15 mg/kg compared with 5, 7.5 and 10 mg/kg (Foreyt, 1988). However, in a field trial to evaluate the efficacy of anthelmintics against natural infected cattle with *Fasciola hepatica* in Turkey, it was shown that the reduction of egg counts was smaller (66.7%) for ABZ compared with rafoxanide (68.2%), triclabendazole (78%) and clorsulon (84.2%) (Elitok et al., 2006). ABZ was also shown to be non effective against natural infection with *Dicrocelium denditricum*, and fairly effective (71%) against *F. hepatica* in sheep (Onar, 1990).

In other animal species ABZ has been used to treat different parasites. In pigs, ABZSO administrated subcutaneous at doses of 15 mg/kg once a day for 8 days, verified that all *T. solium* cysts in muscles were non viable, while 41% of cysts located in the brain were viable (Peniche-Cardeña et al., 2002). In birds, ABZ was determined to be effective against *Raillietina tetragona*, which is one of the most common parasites in domestic fowl (Saeed, 2007).

1.2.5.2. Efficacy in Humans

ABZ is now widely used in large scale treatment programmes for intestinal nematode infection in the human population. Such programmes are promoted by the World Health Organization (WHO), (UNICEF) and the Partnership for Child Development. ABZ has been used for cystic echinococcosis, neurocysticercosis, gastrointestinal nematodes, filariasis and giardiasis. For cystic echinococcosis, albendazole and mebendazole have been the drugs most used in different situation; a) in cases before (four days) an after (one month) puncture, aspiration, injection and re-aspiration (PAIR) b) patients with primary cyst in lungs and liver or with multiple cysts in two or more organs and peritoneal cysts c) for prevention of secondary echinococcosis and d) in combination with praziquantel (WHO, 1996; Kern, 2006). Medical treatment with ABZ alone or with other compounds has been reported with favorable results especially with small and medium cysts (Davis et al., 1989; Singounas et al., 1992; Todorov et al, 1992; Jamshidi et al., 2008; Seckin et al., 2008). Although hydatid

desease reoccurred in the brain (Seckin et al., 2008), the authors suggested that probably the time of medical treatment (3 months) was not sufficient to prevent reoccurrence. In neurocysticercosis, albendazole has been used to treat patients with more efficacy and it is cheaper than the previous drug used, praziquantel (Gracia et. al., 2003; Matthaiou et. al., 2008). Studies on the pharmacokinetic behaviour of benzimidazole antihelmintics have indicated that their efficacy and spectrum of activity could be improved by using procedures which extend and maintain plasma and time concentration beyond that normally achieved by a single drenching. In this context, albendazole has been used in high doses for a long period to treat metacestodes with efficacy in humans (Horton, 2003; Chai et. al, 2004; Matthaiou et. al., 2008). A variety of therapeutic regimes ranging from 15 to 30 mg/kg for periods of 3 to 30 days have been shown to be effective against neurocysticercosis in humans (Cruz et al., 1995; Takayanagui et al., 1992; Medina et al., 1993; Bustos et al., 2006; Rocha et al., 2008). Some controversy was raised about the anthelmintic (ABZ and praziquantel) therapeutic value which has been related to adverse effects such as the reduction of cysts and the subsequent control of seizures (Sotelo, 2004). These disagreements were clarified by a meta-analisis study that showed a superiority of these agents compared with placebo (Del Bruto et al., 2006). Recently, another meta-analysis published, suggested that ABZ was more effective than praziqualtel regarding clinically important outcomes in patients with NCC. Nevertheless, more comparative interventional studies are required to draw safe conclusions about the best regimen for the treatment of patients with parenchymal NCC (Matthaiou et. al., 2008).

The findings in *in vivo* research were supported by a multiplicity of *in vitro* investigations. These vary from single ABZ or ABZSO to combinations with other compounds in different tapeworm genus. When *Tetrathyridia* (larva stage) were exposed to praziquantel and ABZ extensive tissue damage, especially on tegument, adult forms were the most susceptible to drug exposure (Markoski et al., 2006). The minimum exposure time required for activity and effectivity concentration of ABZSO and praziquantel against *T. solium* and *T. crassiceps* cysts were evaluated and results showed that both drugs are time dependent and that ABZSO acts much more slowly and is less potent than praziquantel (Palomares et al., 2004). Polamares et al. (2006), demonstrated changes in ultrastructure of *T. cracisseps* larva when combing ABZSO. Echinococcus cysts were incubated in ABZSO and $ABZSO_2$ demonstrated that both metabolites had a similar effect on ultrastrusctural changes in parasites (Ingold et al., 1999). Activity of ABZ against Echinococcus protoscolicies was investigated under in vitro conditions. Calgar et al. (2008) revealed that ABZ leads to the death of 65% of

protoscolices in the first 5 minutes and 70% in 60 minutes. However, treatment with ABZ against Human Taeniasis showed not be effective in different therapeutic regimes (Chung et al., 1991).

Effective regimes of ABZ in humans have been used for treatment of ascaris, hookworms and *Trichuris trichura* and *Trichinella* (Hanjeet and Mathias, 1992; Dupouy-Camet et al., 2002). However, ABZ showed low efficacy against *T. trichuris* in school-age children in Uganda (Olsen et al., 2009). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) recommends albendazole in combination with other antifilarial drugs. Results and efficacy evaluation of ABZ alone or in combination with diehtilcarbamazine to reduce microfilaria, was diverse and further research is necessary to make conclusions (Critchley et al., 2005)

ABZ proved to be an alternative for giardia infection treatment (Liu et al., 1996). In vitro, ABZ inhibits the growth of trophozoites of *Giardia intestinale* and their adhesion to culture intestinal epithelial cells and disturbs the activity of microtubules and microribbons in the trophozoite adhesive disk. An in vitro study indicated that ABZ metabolism occurs in trophozoites of *G. duodenalis* (Oxberry et al., 2000) ABZ at the dose of 400 mg per day for 5 days, cured 95% of infections in children in Bangladesh (Hall and Nahar, 1993)

1.2.6. Adverse effects

The main adverse effect of benzimidazole compounds involves their embryonic and teratogenic effect. Albendazole has low acute toxicity when given orally to mice, rats, hamsters, guinea pigs and rabbits (EMEA, 1997). The main congenital defects identified in lambs were skeletal malformation, occurring mainly in the long bones, pelvis, joints and digits, and vascular malformation (Navarro et al., 1998). This vascular malformation occurs because the drug and the metabolite, ABZSO, have the capacity to cross the placenta barrier and reach concentrations in the sheep and rat embryo (Cristòfol et al., 1995; 1997, and Capece et al., 2002; 2003). In humans, ABZ is quite safe, but when used in high doses for a long period it can cause elevated levels of liver enzymes and rare cases of leucopenia, neutropenia, aplastic anemia (Horton, 2003; Meneses da Silva, 2003; Opatrny et al., 2005; Akal et al., 2008).

Albendazole has a big safety margin and no signs of toxicity were observed when ABZ was given to goats orally at doses of 75 mg/kg (Foreyt, 1988).

AIM OF THE STUDY

2. Aim of the study

Livestock production in Mozambique is an important agriculture sub-sector, chiefly constituted of 1.272.245 cattle, 3.900.507 goats, 220.516 sheep and 1.339.689 Pigs (INE, 2011). Goats are the main ruminant species in developing countries, including Mozambique, where the major breed distributed throughout the country is the "Landim" which is also designated as Portuguese Landrace (Gall, 1996). According to the National Statistics Institute (INE, 2011), goats are almost entirely (97.7%) managed by smallholder farmers and they are mainly used for meat production and provide extra earnings (Morgado, 2000). They are an important resource for the rural populations since they need smaller investments, have a shorter production cycle, faster growth rates and greater environmental adaptability than cattle. In terms of health constraints, parasitic diseases are considered the most important causes of goat morbidity and mortality. Infections with *Taenia multiceps* cysts are common and are regarded as an important economic and production constraint.

Clinical diagnostic of coenurosis is very difficult and there is no specificity due to the variability of symptoms, which differ according to the size and location of cysts on the central nervous system. Imagiology and post mortem examination has been used to confirm uspected infections in the central nervous system. However, post mortem examination is of very low sensitivity for infections located in muscular tissues, which are normally not included in meat inspection routines. The development of other diagnostic tools, especially those suitable for *in vivo* diagnosis such as serological assays, would be of practical interest.

As coenurosis is a neglected zoonosis, very little has been done regarding disease prevention and control. The general lack of local information on the *T. multiceps* larva led to the development of a series of studies, which are presented in this thesis. These studies were carried out by the Veterinary Faculty of Universidade Eduardo Mondlane (Maputo, Mozambique) in partnership with the Veterinary Faculty of Universidad Autonoma de Barcelona, Department of Pharmacology Therapeutics and Toxicology.

This study aimed at addressing some of the gaps in knowledge regarding *Taenia multiceps* infection in Mozambican goats. The study intends to provide better understanding of some biological parameters of *T. multiceps* and evaluate the therapeutic response of different dosage regimes of albendazole in *T. multiceps* infected goats.

The specific objectives were:

- To determine prevalence of parasites in goats using the the animals routinely sacrified in slaugtherhouse.
- To determine prevalence, morphological characteristic and predilection sites of *Taenia multiceps* cysts in naturally and experimentally infected goats
- Molecular characterization of the gene sequence of CO1 (cytochrome c 1 subunit) and the ND1 (NADH dehydrogenase 1) mitochondrial genes of *T. multiceps* from goats in Mozambique.
- Characterization, by SDS-PAGE, larval antigens of *Taenia multiceps* and determine, by Western Blotting, their specificity
- Analysis the pharmacokinetics profile of ABZ and metabolites after a single dose administration of ABZ to healthy goats.
- Analysis the pharmacokinetics profile of ABZ and metabolites after a different single dose of ABZ in Taenia multiceps infected goats.
- Analysis the pharmacokinetics profile of ABZ and metabolites after an administration of a multiple doses of ABZ in Taenia multiceps infected goats.
- Determine the efficacy of a single or multiple doses of albendazole in animals, two or five month post-infection.

MATERIAL AND METHODS

3. Material and Methods

3.2. Studies, funds and ethical issues

In order to achieve the aims of this program, three different studies were carried out, namely: i) a baseline survey of goats naturally infected with parasites at Tete Municipal abattoir, Mozambique; ii) characterization of *Taenia multiceps* larva in goats iii) a pharmacokinetic parameters assessment of albendazole in healthy and *T. multiceps* infected goats iv) a study on efficacy of albendazole in goats experimentally infected with *T. multiceps*. All studies were developed at the Veterinary Faculty, Eduardo Mondlane University in Mozambique. For the baseline sample collection and experimental *Taenia multicpes* infection and treatment project: "*Eficácia do albendazol contra Coenurus cerebralis em caprinos*", and for pharmacokinetic parameters project; "*Avaliação da eficácia de fármacos anti-parasitários em caprinos e bovinos*". The pharmacokinetic and antigenic characterization studies were undertaken in Spain at the Department of Pharmacology Therapeutics and Toxicology and at the "Center de Recerca en Sanitat Animal (CReSA), Universidad Autonoma de Barcelona (UAB), Barcelona and at the ¨Instituto de Salud Carlos III" (ISCIII), in Madrid. The molecular analysis of *Taenia multiceps* was conducted at the ¨Instituto de Higiene e Medicina Tropical" (IHMT), "Universidade Nova de Lisboa¨, Portugal. This project and all studies involving animals were approved by the Scientific Committee of the Veterinary Faculty, Eduardo Mondlane University (EMU), Maputo, Mozambique. The animals were maintained under guidelines of Good Clinical Practices (GCP) in appropriate facilities at the Veterinary Faculty, EMU.

3.2. Baseline abattoir survey and sampling collection

One hundred and forty nine goats, representing 88.2% of the total animals slaughtered during the sampling period (one week), were screened for *Taenia multiceps* larvae. The goats were from all districts of Tete Province; 87.2% (130/149) were males with 1 to 3 years of age.

Sampling for cysts was done according to the methodology described below and the cysts were preserved at 4^{o}C or 70% ethanol and taken to the parasitology laboratory at the Veterinary Faculty, EMU in Maputo, Mozambique.

The collection of samples was performed at a slaughterhouse where subcutaneous and muscle (n=149), brain (n=142) and gastrointestinal tract tissues (n=115) were examined for the presence of cysts, following routine inspection procedures, consisting of visual examination

of dressed carcass, palpation of internal organs (lungs, heart and liver) and palpation and incision of retropharyngeal and mesenteric lymph nodes. The skull was opened and the brain removed and examined for the presence of cysts. The location of detected cysts and lesions were recorded. Identification of *T. multiceps* cysts was based on morphological characteristics, including presence of a clear fluid and of various protoscoleces on the cyst membrane. All detected *T. multiceps* cysts were carefully removed from host tissues and washed with phosphate buffered saline (PBS) and preserved at 4 ^{o}C or in 70% ethanol for identification and molecular characterization respectively.

Additionally, 110 faecal samples were collected from the same slaughtered goats. The samples were then examined using qualitative simple test tube flotation according to Hansen and Perry, (1994). Briefly, approximately 3 g of faeces were mixed in 50 ml of saturated NaCl solution. The solution was gently mixed and sieved. A test tube was then filed with the suspension to the top, until a slight positive meniscus was formed. The tube was carefully covered with a cover slip and allowed to stand for 20 minutes. The cover slip was then mounted on a microscope glass slide and observed under a light microscope (Zeiss®), using 10X objective. All parasitic stages (eggs, larva and oocysts) were identified based on morphological characteristics according to Soulsby (1992).

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3.3. *Taenia multiceps* Charactrization

3.3.1. Molecular characterization

3.3.1.1. Parasites collection

From a total of 22 *T. multiceps* naturally infected goats, 32 cysts were recovered and15 (4 from the brain and 11 from muscular and subcutaneous tissue) were used for molecular characterization of the parasite. After removal from the surrounding tissue, the cysts were washed in PBS and conserved in 70% ethanol.

3.3.1.2. DNA Extraction

DNA was extracted from 50 µg of cystic material, using the Jet Quick Tissue DNA spin Kit/50 (Genomed®) according to the manufacturer´s protocol. Genomic DNA was quantified using the GeneQuant RNA/DNA Calculator Biochrom.

3.3.1.3. Polymerase chain reaction (PCR) and DNA sequencing

Two mitochondrial genes encoding for cytochrome *c* oxidase subunit I, and NADH dehydrogenase subunit 1 (COI and ND1, respectively) were amplified by polymerase chain reaction (PCR) as described by Gasser et al. (1999a), using 20 ng of genomic DNA. PCR product for COI (~450 bp) was amplified with the primers JB3 (5`- TTT TTT GGG CAT CCT GAG GTT TAT – 3`) and JB4.5 (5'- TAA AGA AAG AAC ATA ATG AAA ATG – 3`), while ND1 (~500bp) were amplified with primers JB11 (5`- AGA TTC GTA AGG GGC CTA ATA – 3') and JB12 (5`- ACC ACT AAC TAA TTC ACT TTC – 3') (Bowles et al.,

1992). PCR reactions (50μl) were performed in 10 mM Tris-HCl, pH 8.4; 2 mM $MgCl_2$, 250μM of each dNTP (Bioline®), 25 pmol of each primer, and 2 unit *Taq* polymerase (Invitrogen®) under the following cycling conditions: 94°C, 5 minutes (initial denaturation), followed by 30 cycles of 94°C (30 sec), 55°C (30 sec) and 72°C (30 sec), and a final extension at 72°C for 5 min. Amplified products were analysed by agarose gel electrophoresis using the 100 bp DNA ladder (Invitrogen®). Specific PCR amplicons were purified and sequenced (STAB-VIDA, Portugal).

3.3.3. Data analysis

The prevalence of *T. multiceps* larvae at the abattoir and the parameters associated with the experimental infections in goats were compared, using a chi-square test with level of significance at $P<0.05$. The software SPSS 13.0, SPSS Inc., Chigago, II was used to analyse data.

Generated sequences were analysed and compared with the genebank sequences from different Taeniidae species, such as: *T. multiceps*, *T. madoquae*, *T. serialis*, *T. solium*, *T. ovis*, *T. saginata*, *T. asiatica*, *T. polycantha*, *T. hydatigena*, *T. regis* and *T. pisisformis* (Table 3). DNA sequences were aligned using CLUSTALW (available at http://www.ebi.ac.uk/clustalw/), and manually stripped for gaps. Phylogenetic inference analyses were conducted using MEGA version 2, from pairwise genetic distance matrices calculated with the Kimura two-parameter algorithm. Tree topologies were inferred by the neighbour-joining method and the robustness of the obtained trees evaluated by bootstrap analysis of 1000 re-samplings of the sequence data.

Table 3 - List of Taenidae genomic sequences compared to *Taenia multiceps* in the sudy

Species	Target gene	Size (pair bases)	Genebank accessing number	Geographic área	References
T. multiceps	ND1	471	DQ077820	Italy	(Varcasia et al., 2006)
	ND1	471	AJ239104	UK	(Grasser et al. 1999b)
	ND1	471	AY669089	Italy	(Varcasia et al., 2006)
	ND1	471	DQ309770	Italy	(Varcasia et al., 2006)
	ND1	462	HM1014	Iran	(Oryan et al., 2010)
	CO1	445	DQ321830	Italy	(Varcasia et al., 2006)
	CO1	445	DQ309767	Italy	(Varcasia et al., 2006)
	CO1	444	EF393620	Turkey	Unpublished *
	CO1	445	DQ309768	Italy	(Varcasia et al., 2006)
	CO1	445	DQ309769	Italy	(Varcasia et al., 2006)
	CO1	445	DQ309767	Italy	(Varcasia et al., 2006)
	CO1	396	HM101469	Iran	(Oryan et al., 2010)
	CO1	396	FR873148	U. E. U.	Unpublished
	CO1	366	JQ710577	Iran	(Rostmami et al., 2013)
	CO1	445	HM143882	Turkey	(Avcioglu et al., 2011)
	CO1	445	HM143886	Turkey	(Avcioglu et al., 2011)
	CO1	445	HM143884	Turkey	(Avcioglu et al., 2011)
	CO1	1623	JX507225	China	Unpublished
	CO1	1623	JX535567	China	Unpublished
T. krabbei	ND1	488	EU544625	Finland	(Lavikainen et al., 2008)
	ND1	488	EU544629	Finland	(Lavikainen et al., 2008)
	Cox 1	395	JF261325	Finland	(Lavikainen et al., 2011)
T. madoquae	ND1	488	AM503343	Kenya	(Zhang et al., 2007)
	ND1		AB73726	Kenya	(Nakao et al., 2013)
	Cox1	396	AM503325	Kenya	(Zhang et al., 2007)
T. serialis	ND1	530	DQ401137	France	(Collomb et al., 2007)
	ND1	13688	AB731674		(Nakao et al., 2013)
	Cox1	378	DQ401138	France	(Collomb et al., 2007)
T. solium	ND1	530	EF076753	India	Unpublished **
	Cox 1	444	EF076752	India	Unpublished **
T. ovis	ND1	471	AJ239103	New Zeeland	(Grasser et al. 1999b)
	ND1	13707	AB731675		(Nakao et al., 2013)
T. saginata	ND1	488	AM503345	Kenya	(Zhang et al., 2007)
	ND 1	13670	AY68274		(Jeon et al., 2007)
	Cox1	396	AM503327	Kenya	(Zhang et al., 2007)
	Cox 1	924	JN986702	Thailand	(Anantaphruti et al., 2013)
T. asiática	ND1	471	AJ239108	Taiwan	(Grasser et al., 1999b)
T. polyacantha	ND1	488	DQ408420	Finland	(Trachel et al., 2007)
T. hydatigena	ND1	530	DQ995654	India	Unpublished **
	Cox 1	444	DQ995656	India	Unpublished **
T. regis	ND1	488	AM503346	Kenya	(Zhang et al., 2007)
	Cox1	396	AM503328	Kenya	(Zhang et al., 2007)
T. psiformis	ND1	474	AJ239109	Australia	(Glasser et al. 1999b)
T. taenuiformis	ND1	471	AJ239101	Australia	(Glasser et al. 1999b)
	Cox1	444	EF090612		Unpublished **
T. crassiceps	Cox 1	825	AB033411		(Nakao et al., 2000)

3.3.2. Antigen Characterization

3.3.2.1. Antigen preparation

Taenia multiceps cysts were dissected from muscles and brains of naturally infected goats and used to prepare antigen from the cyst fluid (AgF), membrane and scolex (AgFE) and oncosphere (AgO). Additionally, *T. solium* (Ts) and *T. hydatigena* (Th) larva from naturally infected pigs and goats were collected for preparation of antigen from the fluid, membrane and scolex. Antigens were used for western blot and ELISA.

Fluid antigen (AgF) - A 20 ml syringe was used to retrieve the fluid from the cyst. The fluid was then centrifuged at 4° C for 30 minutes at 5000 rpm (MSE Ministral 300, Sanyo®). The supernatant was collected and centrifuged again under the same conditions. The second supernatant was collected and aliquoted to 2 ml cryopreservation vials and stored at -20 ° C.

Fluid membrane and scolex antigen (Ag FE) (Ts and Th) - Cysts were removed from the host tissue and washed in PBS (pH 7.2), then homogenized in ice, for 10 minutes. After homogenization the tissue suspension was disintegrated using ultrasound (Soniprep 150, Sanyo®) eight times at 8 KHz, with 30 seconds cooling intervals. Each sonication period was of one minute in an ice bath. Sonicated material was centrifuged at 4°C for 30 minutes at 5000 rpm (MSE Ministral 300, Sanyo®), the supernatant was collected and centrifuged again under the same conditions. After centrifugation the supernatant was dispensed as complete homogenate antigen into 2 ml cryopreservation vials and stored at -20 ° C.

Onchosphere antigen (Ag O) - Oncospheres were suspended in RPMI 1640 media (40 000 onchospere/ml) and sonicated at 4°C at 70 Hz (Soniprep 150, Sanyo®) using three periods of one-minute separated by one-minute rest and cooling intervals. The onchosphere preparation was centrifuged at 28 000 g for 30 minutes at 4°C and the supernatant was then collected and stored in 2 ml cryopreservation vials at -20°C until used for ELISA standardization.

Antigen protein content was determined using:

- Veterinary Faculty, UEM, Mozambique: the ultraviolet absorption (280 nm).
- **ISCIII, Madrid, Spain**: Previous to protein quantification a protease inhibitor was used at 1:200 dilution. Biocinchoninic acid (BCA) for the calorimetric detection and quantification of total protein (BCA Protein Assay Kit, Pierce®) was used.

- **CReSA, UAB, Spain**: Before quantification a protease inhibitor was added to each antigen at 1:100 dilution. Protein were quantified using a commercial protein quantification kit EZQ (Invitrogen®) a fluorescence-based test was used according to manufacture direction.

3.3.2.2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), dot blot and Western Blot

Antigen was separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970), with a gel (5% stacking + 10% separating) running at constant amperage of 0.03 for 2 hours . Each antigen was diluted in order to get approximately 1288 μg/ml of protein. Proteins were visualized after staining with Coomasie Blue (Sigma-Aldrich®). The protein molecular weights were determined by comparing with molecular weight standards (BIO-RAD®). Antigenically active components among SDS-PAGE resolved bands were detected by Western blotting. After SDS-PAGE, the proteins were electrophorectically transferred onto nitrocellulose paper (Protran, Whatman®), using a semi dry transfer apparatus (Fastblot, Biometra®, Trans-Blot SD, BioRad® and NuPAGE, Invitrogen®). The transfer process was conducted for 1 hour, using constant voltage 180V. Nitrocellulose containing transferred samples were incubated overnight in Tris Buffered Saline (TBS) 5% non-fat milk and then rinsed three times, 5 minutes each with PBSwith 0.05% tween 20 (washing solution) or washing with TBS pH7.6 twice with 25 ml with short agitation following 15 min 100 ml (100rpm) and the last three times for 5 min each 100 ml (100rpm) .

In order to achieve best results for Western blots a dot blot technique was performed with a checkboard pattern to determine the optimal antibody and conjugate concentration pair.

The blocked nitrocellulose membrane was incubated for one hour with diluted sera (1:100 and 1:400) in 1% non fat milk in PBS. Following three washes with PBS 0.05% Tween 20 or washing with TBS pH7.6 twice with 25 ml with short agitation following 15 min 100 ml (100rpm) and the last three times for 5 min each 100 ml (100rpm) to remove the unbound antibodies. Nitrocellulose membrane was then incubated for one hour in horseradish peroxidase conjugated rabbit anti goat IgG (ABD, Serotec®) diluted (1:1000) in 1% non fat milk PBS. Unbound conjugate was removed by three washes with PBS with 0.05% tween 20 before the addition of the substrate solution containing 3,3' – Diaminobenzidine (DAB,

Sigma-Aldrich®) for 30 minutes or chemiluminescent (Lumigen TMA 6, LUMIGEN®) for 10 min in dark. The reaction was stopped by addition of distilled water to the nitrocellulose membrane. Antigenically reactive bands stained reddish-brown. For chemioluminescent samples western blot development were done using fluorchen HD2 (Alpha, Innotech®).

3.3.2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA test has been performed by the use of negative sera panels from 2 adult goats from UAB and 16 animals not infected with *T. multiceps* according to the diagnosis in the Tete slaughterhouse but with others parasitic infection such as *Stilesia hepatica* (n=29), *Cysticercus taenuicolis* (n=7), gastrointestinal nematodes (n=16), coccidian (n=15) and *Moniezia* spp (n=2). Additionally, sera from 5 experimental infected *T. multiceps* goats with cysts varying from 0 to 15 were tested from 0 to 165 days post infection.

Antibody detection (Ab-ELISA)

Antibody response against *T. multiceps* larvae was evaluated on serum samples from experimental and naturally infected goats using 96 wells polystyrene microplate (MaxiSorp, NUNC®). Antigen (AgFE) was diluted (1:200) in 50 mM carbonate buffer pH 9.6 and incubated overnight at 4°C, washed twice with PBS with 0.05% Tween 20. Blocking was done with 200 µl in each well of casein buffer for 60 min at 37°C. After washing as previously described, diluted (1:200) serum samples were add for 60 min at 37°C. After incubation, excess serum were washed as earlier explained and conjugate horseradish peroxidase rabbit anti goat IgG (BIO-RAD®) diluted (1:1000) in casein buffer for 60 min at 37°C, and washed three times with PBS with 0.05% Tween 20. Subsequently, this was incubated for 30 min at room temperature in dark 100 µl of substrate, 2,2- azino-di-[3-ethyl-benzthiazoline sulfonate] (ABTS) (Sigma-Aldric®) diluted at citrate buffer. Reaction was stopped by adding 100 µl of 1% sodium dodecyl sulphate (SDS) and absorbance was read at 405 nm (ELISA reader, Merk®).

Antigen detection (Ag-ELISA)

Antigen was detected with a HP10 monoclocal antibody (McAb), ELISA was performed as described by Harrison et al. (1989). Polystyrene high protein-binding 96 microplates (MaxiSorp, NUNC®) were coated with, 100 μl to each wells, monoclonal antibody (McAb) HP10 diluted at 1:400 in borate buffered saline (BBS) pH 8.2 and left overnight at 4°C, washed twice with a solution of 0.9% NaCl containing 0.05% Tween 20. After washing,

blocked using 200 μl, in each well, of PBS, pH 7.4 with 1% of bovine serum albumin (BSA) and 0.2% Tween 20 and incubated for 1 hour at 37^{o}C then microplates were washed 3 times as above. One hundred microliter of undiluted serum samples were added for each well and then incubated for 30 min at 37^{o}C. Biotinylated HP10 diluted at 1:400 in PBS pH 7.4 with 0.1% BSA and 0.02 tween 20 (PBST) and incubated 30 min at 37^{o}C and microplates were washed as mention above. Conjugate, streptavidin horseradish peroxidise, diluted at 1:1000 were added (50μl) and left at 37^{o}C for 60 min, washed as above, added 100 μl of substrate 3,3',5,5'-tetramethylbenzidine (TMA, Sigma-Aldrich®) at room temperature for 30 min in dark and reaction was stopped by adding 100 μl 0.2 H_2SO_4 per well. ELISA reader (Merk®) at 450nm was used to determine the optical density.

3.4. Pharmacokinetics studies

3.4.1. Chemicals

To undertake laboratory analyses, the following chemicals were used: i) Albendazole (ABZ) was provided by Hoeschst Marion Roussel (Frankfurt AM, Germany), ii) ABZSO and ABZSO, were supplied by Schering-Plough (Union, NJ,USA ESA) and iii) oxibendazole (OXB) used as internal standard was provided by Basf-Labiana (Terrassa, Spain). Albendazole administrated to treat animals was the oral 10% suspension, ALBENOL -100® from Interchemie, Holland.

3.4.2. Animals and treatment

Pharmacokinetics in healthy animals: Twelve males (6 adults and 6 two-month-olds) and 12 females (6 adults and 6 two-month-olds) local breed, "Landim" healthy goats were used for albendazole pharmacokinetics in single oral doses of 10 mg/kg. Animals came from a commercial farm near Maputo and they were kept indoors from the day before treatment and during the sampling period. Goats were fed with hay and water ad libitum. Health of animals was monitored prior to and throughout the course of the study. Young animals were left in the companion of their respective mothers. Animals were allocated to 4 groups (n=6) according to their sex and age.

Pharmacokinetics in males experimentally infected with *Taenia multiceps*: Twenty-one adult male local breed goats, experimentally infected with *T. multiceps*, were used for albendazole pharmacokinetics of single and multiple doses. Provenience and management conditions of animals were the same as above mentioned. Animals were divided randomly

into two groups of 7 animals each. Goats were treated with oral suspension of ABZ at single doses of 10 mg/kg (Group 10 mg) and 20 mg/kg (group 20 mg) and multiple doses of 10 mg/kg a day during 3 consecutive days. Furthermore, five male goats experimentally infected with T. *multiceps*, under the same conditions above mentioned were used to evaluate the presence of ABZ and its metabolites in brain, muscles and cyst fluid after a single dose of 10mg/kg.

3.4.3. Sampling collection

Blood samples (approximately 3 ml) were collected from the jugular vein into heparinized vacutainer tubes (Bencton & Dickison ®, NJ, USA) prior to the treatment and at 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 42, 48 and 54 h post treatment for the group treated with a single dose. For the group treated with 3 doses (24 hour interval) blood was collected at 0, 0.5, 1, 2, 4, 6, 10, 12, 18, 24, 24.5, 25, 26, 28, 30, 32, 34, 36, 40, 44, 48, 48.5, 49, 50, 52, 54, 56, 50, 60, 64, 68, 72, 78, 84, 90, 96, 102, 108, 114 and 120 h after the first treatment. Plasma was obtained by centrifugation at 3000 g for 20 min and kept at – 20ºC until analysis.

For evaluation of the presence of ABZ and its metabolites, blood samples were taken, as referred to above, prior to the treatment and at 1, 2, 6, 8, 10 and 12 hours after the treatment. Animals were humanely euthanized 12 hours after ABZ administration and approximately 5gr of brain (n=5) and muscles (n=5) and 2 ml of cyst fluid (n=3) were collected and preserved at -20ºC until use.

3.4.4. Analytical procedures to determination of albendazole and metabolites

To quantify the samples, standard curves were prepared with blank plasma samples to which pure standard ABZ, ABZSO and $ABZSO_2$ were added. After adding the internal standard, samples were extracted as described below. A least-squares regression analysis between the chromatographic peak area ratio (ABZSO or $ABZSO_2$/internal standard) and the plasma concentration of the standard samples was used to calculate the amount of ABZSO or $ABZSO_2$ present in the samples.

Plasma extraction was performed according to Capece et al. (2000). Aliquots with collected plasma samples (1 ml) were supplemented with 2.5 μg of OXB, 1 ml of ammonium acetate buffer solution (0.025 mol/l, pH 7.2) and 10 ml of ethyl acetate. This solution was shaken in a vortex for 20 min and centrifuged for 20 min at 2000g. The supernatant (organic phase) was then collected with a Pasteur pipette and transferred to a conical tube. The collected organic

phase was evaporated to dryness under a rotary evaporator in a bath at 37ºC. The dry extract was dissolved in 200 μl of mobile phase (67% of 0.025 mol/l ammonium acetate pH 7.2 and 33% of acetonitrile).

All samples were placed in a cartridge and 50 μl was injected into the high-performance liquid chromatography (HPLC) system with UV detector (HP 1100 Series, Hewlett-Packard GmbH, Waldbronn, Germany). The mobile phase consists of ammonium acetate solution (0.025 mol/l, pH 7.2) and acetonitrile. The solvent system was an acetonitrile-ammonium acetate gradient with the following profile: initial conditions 70:30; 4.0 min to 60:40 proportion; 3 min initial conditions, 70:30. The flow rate was 1.2 ml/min and the absorbance was measured at 292 nm. Under this chromatographic condition the retention time were 2.9 min (ABZSO), 3.3 min ($ABZSO_2$), 4.7 min (ABZ) and 5.3 min (OXB used as internal standard). The quantification limit was fixed at lower value of the calibration curves (0.052g/ml) for all analyses.

Brain and muscles samples before starting with the extraction were minced and ground manually and then the same technique used for plasma samples was applied. Fluid samples were treated the same way as plasma samples.

3.4.5. Pharmacokinetic analyses

Determination of pharmacokinetic parameters was performed using the PK Solutions 2.0.2 software (SUMMIT Research Services, Ashland, USA).

The pharmacokinetic parameters of ABZSO and $ABZSO_2$ were determined using a NON-COMPARTMENTAL MODEL ANALYSIS (Berrozpe et al., 1997) as it provides a framework to introduce and use statistical moment analysis to estimate pharmacokinetic parameters.

Non compartmental methods can be used to determine certain pharmacokinetic parameters without deciding on a particular compartmental model. The basic calculations are based on the area under the plasma concentration versus times curve (zero moment) and the first moment curve (AUMC). The AUC can be calculated as before using the trapezoidal rule. The first moment is calculated as concentration times time (Cp • t). The AUMC is the area under the concentration times time versus time curve. This may be best covered with an example. Consider a drug given both by IV and oral administration. Both the AUC and AUMC were calculated using the trapezoidal rule without making any assumption concerning the number of compartments. The final segment of the AUC curve is calculated as Cp(last)/kel, where kel is the last exponential term (the slowest) calculated from the Cp versus time graph.

Therefore, after the drug is administered, it is absorbed in the body, and will reach a peak plasma concentration, Cmax, at time Tmax. With the curve drug plasma concentration/time we can obtain:

a) Cmax: Peak drug plasma concentration

b) Tmax: Time to peak plasma concentration

c) AUC: Area under the plasma concentration time curve

d) MRT: Mean residence time

e) t1/2: elimination half-life.

Peak drug concentration (Cmax) and the time were the maximum peak is observed (Tmax) are related to each animal. These are parameters which are related to absorption and drug formation or their metabolites.

The Area Under the Curve (AUC), is the zero statistic moment corresponding to the concentration-time curve. It is estimated by the trapezoid calculation rule from 0 h to last time (AUC(0-t)) following the equation:

$$AUC_{t_1-t_2} = \frac{C_1 + C_2}{2} \times (t_2 - t_1)$$

$$AUC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2} (C_i + C_{i+1})$$

The **Mean Residence Time** (MRT) can be used to estimate the average time a drug molecule spends in the body. It can also be used to help interpret the duration of effect for direct-acting molecules.

For drugs with linear kinetics, the amount in the body is proportional to the concentration in plasma at all time points. By making these substitutions, we can arrive at the following for MRT calculations:

$$MRT = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} = \frac{AUMC}{AUC}$$

where AUMC, the area under the first moment curve or the curve of concentration.time versus time is calculated using the following equation:

$$AUCM = \sum_{0}^{t} \frac{C_1 t_1 + C_2 t_2 + \cdots + C_n t_n}{n} \times (t_n - t_{n-1})$$

The terminal half-life($t_{1/2}$) is the time required for plasma/blood concentration to decrease by 50% after pseudo-equilibrium of distribution has been reached; then, terminal half-life is computed when the decrease in drug plasma concentration is due only to drug elimination, and the term 'elimination half-life' is applicable.

The decay of a drug following first-order pharmacokinetics being exponential, the terminal half-life is obtained from:

$$t_{1/2} = \frac{0{,}693}{k_{el}}$$

where 0.693 is the natural ln 2 and k_{el} the slope of the terminal phase.

3.4.6. Statistical analysis

The pharmacokinetic parameters calculated for each molecule were compared between sexes and ages by means of nonparametric Mann-Whitney U test. Repeated-Measure ANOVA, student T test and Paired-Samples T test were used to compare kinetic parameters of ABZSO and $ABZSO_2$ within each administration (in multiple doses). Plasma concentrations in different groups were compared by Repeated-Measure ANOVA. These calculations were performed using SPSS 13.0, SPSS Inc., Chigago, Il, USA. In both tests, significance was accepted when $p<0.05$.

3.5. Experimental infection

3.5.1. Animals

Six 2-month-old mixed breed dogs, (3 for each experiment) from the same litter were selected for experimental infection with cysts of *T. multiceps*. Dogs were housed in individual kennels at the Eduardo Mondlane University (EMU) Veterinary Hospital. Animals and were fed a standard commercial diet, supplied with clean water *ad libitum* and were subjected to the following prophylactic schedule established at the EMU Veterinary Hospital:

i. Two doses of multiple vaccines against common viral diseases (Distemper-MLV, Adenovirus Type 2-MLV, Parainfluenza-MLV, Parvovirus-MLV) at 2 and 3 months old;

ii. Rabies vaccination at 3 months old

ii. Two deworming treatments: the first at the arrival at the Veterinary Hospital and the second one month before infection with multi-spectrum anthelmintic composed by pyrantel, oxantel and praziquantel (Canex-4®).

The health of all dogs was routinely monitored throughout the period of study.

Fifty-seven (28 for experiment 1 and 29 for experiment 2) 8-18 month-old *Landim* male goats, were purchased from *T. multiceps*-free farms and used in this study. The farms were considered free from *T. multiceps* taking in account the historical records from the Mozambican Department of Veterinary Services. Animals were housed at the Veterinary Faculty, EMU campus, under good clinical practice (GCP) and maintenance diet based on hay and grain with water supplied *ad libitum*. The adaptation period, from purchase to infection, was four months.

3.5.2. Experimental infection procedures

T. multiceps cysts collected from naturally-infected goats at Tete municipal abattoir were immediately processed after collection by removing the tissue surrounding the cyst. This was followed by washing the cysts in phosphate buffer saline (PBS) pH 7.3 and storing them in a sterile recipient kept at 4°C until experimental infection to dogs.

At 4-months of age, each dog was orally infected with approximately 100 scoleces of *T. multiceps*. Patency was determined by collecting faecal samples from the infected dogs once a week to detect Taeniidae eggs, using McMaster flotation technique (Hansen and Perry, 1994). Dogs were euthanized with 20% pentobarbital (Eutha-Naze®) at a dose of 200 mg/kg, after the parasite had reached patency. *Taenia multiceps* adult parasites were removed from the intestine and gravid proglotids were harvested to recover the eggs. Standard operating procedures regarding the safety of researchers were strictly followed at all stages of the experimental infection. Kennels were kept isolated and the access was restricted only to the researchers. All faeces from the infected dogs were incinerated from day one post infection.

Gravid proglotids of *T. multiceps* were crushed in 2.5% potassium dichromate solution to release the eggs and they were kept in a refrigerator at 4°C before being subjected to the hatching process. *In vitro* hatching was performed using sodium hypochlorite. Briefly, eggs were centrifuged at 1000 rpm for 5 minutes and then 0.5ml of sodium hypochlorite (0.5% of

sodium hypochlorite in normal saline) and 10 ml of normal saline were added to the sediment. The sediment, containing the hatching eggs, was washed three times in normal saline and counted (Lightowlers et al., 1984; Negita and Ito 1994; Takemoto et al., 1995; Wang et al., 1997). Each goat was orally infected with 3000 viable eggs, using cellulose and amide bolus. Steps of experimental infection of goats are shown in **Figure 6 (A and B).**

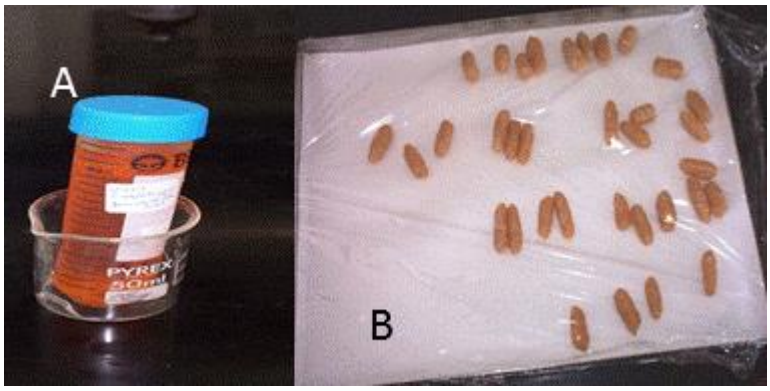

Figure 6 - Experimental infection: *Taenia multiceps* eggs in solution of 2.5% potassium dichromate (A), and cellulose and amide bolus with viable eggs inside (B)

3.5.3. In vivo efficacy of albendazole against *Taenia multiceps* larvae in goats

3.5.3.1. Experimental design

Fifty seven (57) male goats were infected orally with approximately 3000 *T. multiceps* hatched eggs in cellulose and amide bolus and assigned randomly to 5 groups of 8 animals each and one group of 17 animals. For treatment, albendazole (ALBENOL-100® (ABZ), oral suspension- Interchemie, Holland) was administrated orally. Two months post infection, the animals of Group 1 (G1) were treated with ABZ at doses of 10 mg/kg/3 days at 24 hrs interval, group 2 (G2) received a single dose of 10 mg/kg, group 3 (G3) a single dose of 20 mg/kg and group 4 (G4) was the control for the two-month-old cysts. Five months post infection, Group 5 (G5) was treated with ABZ at a dose of 10 mg/kg/3 days and group 6 (G6) was used as control (**Table 4**).

Table 4- Treatament protocols with albendazole for goats infected with 3000 eggs of *Taenia multiceps*

Group (dose)	Number of goats	Time of treatment (months post infection)	Dosage regime
G1	8	2	10 mg/kg/day/3 days interval 24 h
G2	8	2	10 mg/kg/day single
G3	8	2	20 mg/kg/day single
G4	17	2	Control for two months
G5	8	5	10 mg/kg/3 days interval 24 h
G6	8	5	Control for five months

3.5.3.2. Cyst burden assessment

Ten days after treatment, all animals were euthanized. At necropsy, the skeletal muscle from superficial to deep layers of head, neck, thoracic member, thoracic wall, abdominal wall, lumbar region and pelvic member, brain, heart, lungs, diaphragm, liver, kidney and mesentery tissues of the carcass were removed for inspection for *T. multiceps* cysts. Localization of *T. muliceps* cysts was determined by direct observation and the anatomical region was determined according to anatomic classification (Popoesko, 1984; Popesko et al., 1992). Each cyst was measured with a ruler and detected cysts were removed from the tissue and then washed with phosphate saline buffer (PBS) pH 7.3 and the morphology and movement were observed and recorded. The washed cysts were stained with methyl blue according to Casado et al. (1986). Briefly, cysts were incubated for 10 minutes in a solution of 10% methyl blue followed by washing with PBS for 10 minutes. Cysts were considered viable when they showed undulation movements, intact semi-transparent membrane, transparent cyst fluid and negative to methyl blue staining. Cysts were considered non-viable when they presented changes in cyst membrane and fluid, no movements and positive to a methyl blue staining. Furthermore, cyst scoleces were counted under stereomicroscope.

3.5.3.3. Data analysis

Statistical Package for Social Science (SPSS, ver. 13, USA) was used to compare cyst viability between control and treated groups at 2 and 5 months post infection and between treated groups using different doses. A chi-square test was used in both cases and $p<0.05$ was considered as statistically significant.

RESULTS

4. Results

4.1. Baseline abattoir survey

4.1.1. Routine inspection

Larval stages of *Taenia hydatigena* and *Taenia multiceps* as well as intestinal nodules due to *Oesophagostomum spp*. were observed, during direct gastrointestinal tract observation. *Oesophagostomum spp*. nodules were the most prevalent (87%) parasitic condition. Mixed infections, involving *T. hydatigena* larvae and *Oesophagostomum spp*. nodules were detected in 65 (56.5%) animals and two goats (1.7%) presented a mixed infection with *Taenia multiceps* larvae and *Oesophagostomum spp*. nodules. **Table 5**, summarizes the main parasitological macroscopic observations on parasites detected in the gastrointestinal tract of goats at Tete abattoir.

Table 5 - Macroscopic detection of parasites in the gastrointestinal tract goats slaughtered at Tete abattoir

Parasite/lesion	Number Observed	Number positive	Percentage positive
Taenia hydatigena larvae	115	72	62.5
Taenia multiceps larvae	115	2	1.7
Oesophagostomum spp. nodules	115	100	87

From 142 livers inspected at the abattoir, 24 (16.9%) and 9 (6.3%) were positive to *Stilesia hepatica* and *Taenia hydatigena* larvae, respectively.

4.1.2. Coprological analysis

All faecal samples (n=110) were positive for at least one order and three parasitic genera. Strongylida eggs had the highest prevalence (99.1%) and the lowest was found for *Moniezia spp*. (14.5%). *Strongyloides spp* eggs and *Eimeria spp*. oocysts were also observed. The occurrence of gastrointestinal parasites in slaughtered goats is shown in **Table 6**.

Table 6 - Parasites detected by faecal flotation in goats slaughtered at Tete abattoir.

Parasite	Number positive	Percentage
Strongylida	107	99.1
Strongyloides spp.	11	28.2
Moniezia spp.	16	14.5
Eimeria spp.	98	89.1

Single infections were found in 11 (10%) faecal samples and the other 99 (90%) samples presented different types of mixed infections. The more frequent parasitic associations were Strongylida with *Eimeria spp*., being the least common mixed infection represented by Strongylida and *Moniezia spp.* (**Table 7**).

Table 7 - Ocurrence of mixed infections on the faecal samples

Parasitic association	N	%
Strongylida+ *Eimeria spp*.	54	54.5
Strongylida + *Strongyloides spp*.+ *Eimeria spp.*	26	26.3
Strongylida + *Moniezia spp*.	1	1.01
Strongylida + *Eimeria spp*. + *Moniezia spp*.	11	11.1
Strongylida + *Strongyloides spp*. + *Eimeria spp*.+ *Moniezia spp.*	4	4.04
Strongylida + *Strongyloides spp*.	3	3.03
TOTAL	99	

4.1.3. Morphological description of *Taenia multiceps* larvae from natural and experimental infections

The overall prevalence of *T. multiceps* cysts in abattoir-slaughtered goats, through meat inspection, was 14.8% (22/149) and that of experimentally infected goats were 78.6% (22/28) and 82.8% (24/29) for group one and two respectively.

Table 8 - Ocurrence of *Taenia multiceps* cysts (*Coenurus cerebralis*) at various anatomical locations in abattor-slaughtered and experimental infected goats

Location	Prevalence of cysts (%)		
	Abattoir-slaughtered N (% infected)	**Experiment 1 N (% infected)**	**Experiment 2 N (% infected)**
Muscle+subcutaneous tissue*	149 (9.1)	28 (82.0)	29 (75.0)
Mesentery	115 (7.1)	28 (0.0)	29 (4.5)
Lungs	149 (0)	28 (1.0)	29 (2.3)
Diaphragm	115 (0)	28 (5.0)	29 (2.3)
Heart	149 (0)	28 (7.0)	29 (4.5)
Overall Prevalence	22(14.8)	22 (78.6)	24 (82.8)

N = Sample size; Experiment 1 = 10 weeks post-infection; Experiment 2 = 22 weeks post-infection; *Muscle and subcutaneous tissue included muscles from the neck region, thoracic region, abdominal wall, lumbar and pelvic region.

The muscle and subcutaneous tissue were the most common anatomic location of cysts in both the naturally and experimentally (Group one and two) infected goats with a prevalence of 9.1%, 82% and 75% respectively and this was followed by the brain in the abattoir-slaughtered group (8.5%), and Group 2 (11.4%) and the heart (7.0%) and diaphragm (5.0%) in Group 1. A low number of cysts were observed in the brain (5% for experiment 1 and 11.4% for experiment 2). Overall, *T. multiceps* cysts were distributed to a minimum of five anatomical sites in the experimentally infected goats whilst they were only observed at three sites in the naturally infected goats. It is worth mentioning that muscle and subcutaneous tissue included muscles from the neck, thoracic, lumbar and pelvic region and abdominal wall. A significant difference (p 0.05) was observed between the number of cysts observed in the brain of abattoir-slaughtered goats and experimentally infected animals with the abattoir-slaughtered animals having more cysts in the brain. There was no significant difference ($p >$ 0.05) in the number of cysts observed in the muscles and subcutaneous tissue between the two groups.

The morphological characteristics of the *T. multiceps* cysts from naturally and experimentally infected goats were similar. Cysts were composed of a hyaline membrane with many scoleces on the inner surface and filled with a transparent fluid of varying volume. In both cases cysts which were located outside of the brain were surrounded by a thick host membrane (**Figure 7 and 8**).

In the naturally infected group, animals with one cyst were more frequent in both muscular and subcutaneous tissues 11 (73.3%) and brain 9 (75%) and few animals were found to harbour two or more cysts. In the experimentally infected groups, animals with two cysts (11

animals) were the most common and only three goats had 10, 12 and 16 cysts each respectively. Size of larvae and number of scoleces per cyst increased with the age as observed at 10 and 22 weeks post-infection (**Table 9**). Young cysts of 10 weeks of age had fewer scoleces (51.7±27.7) than the older ones of 22 weeks (92.2±48.7).

Table 9 - Size of *Taenia multiceps* cyst (*Coenurus cerebralis*) and number of scoleces per cyst in experimentally infected goats at 10 and 22 weeks post-infection

	Mean length (range) and width (range) of larva at 10 and 12 weeks post-infection		
Time of slaughter post-infection	N	Mean Length ± sd (Min-Max)	Mean Width ± sd (Min-Max)
10 weeks	66	2.1±1.06 (0.1-5.8)	1.4±0.75 (0.1-5.8)
22 weeks	46	3.38±1.4 (1.0-6.8)	2.44±1.12 (1.0-6.8)
	Mean number (range) of scoleces per goat at 10 and 12 weeks post-infection		
	N	Scoleces	
10 weeks	83	51.73±27.7 (6-181)	
22 weeks	36	92.22±48.71 (18-206)	

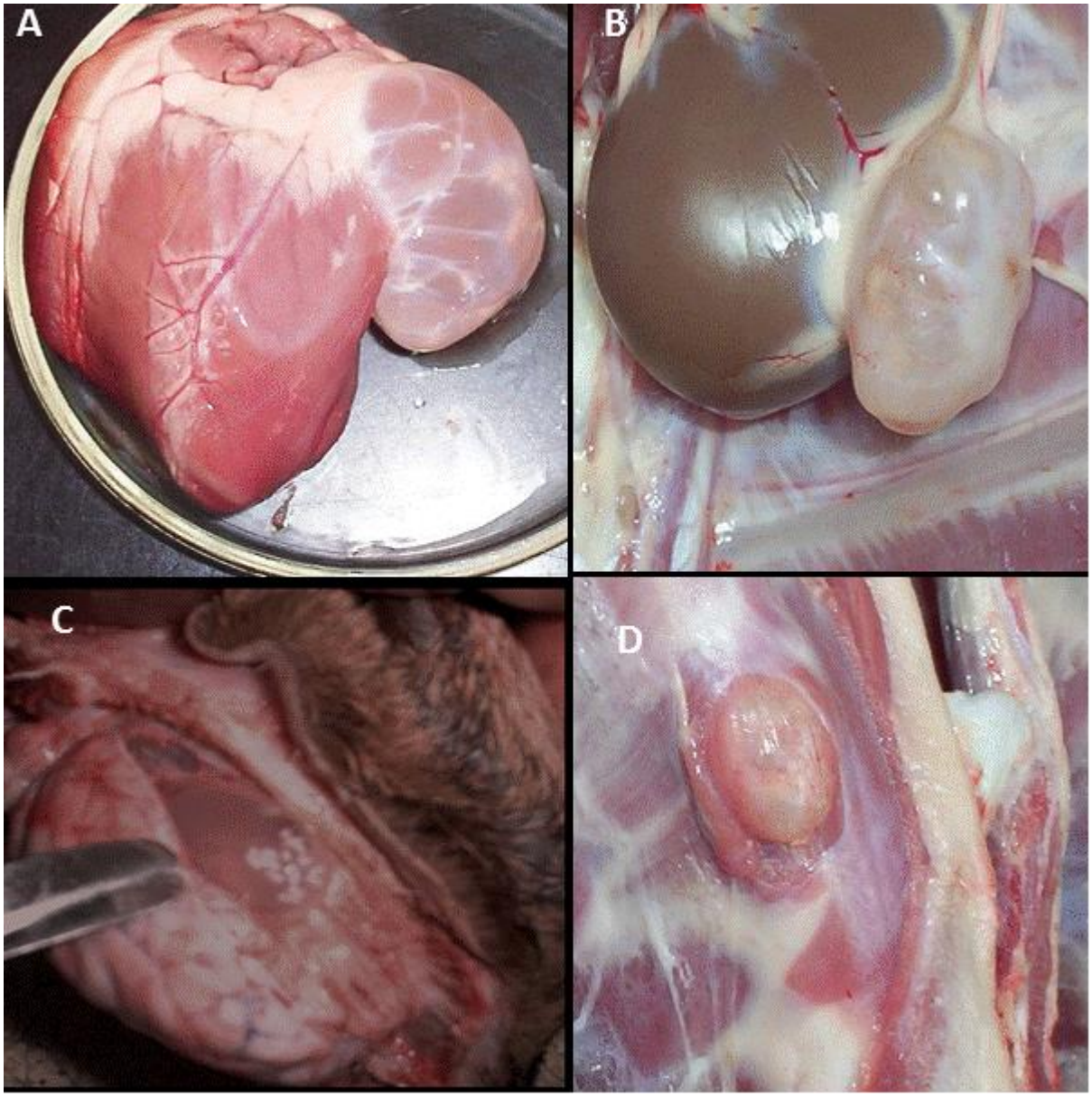

Figure 6- Naturally infected goats with *Taenia multiceps* cyst, at Tete abattoir. (A) heart (B) suprarenal (C) brain and (D) intercostal

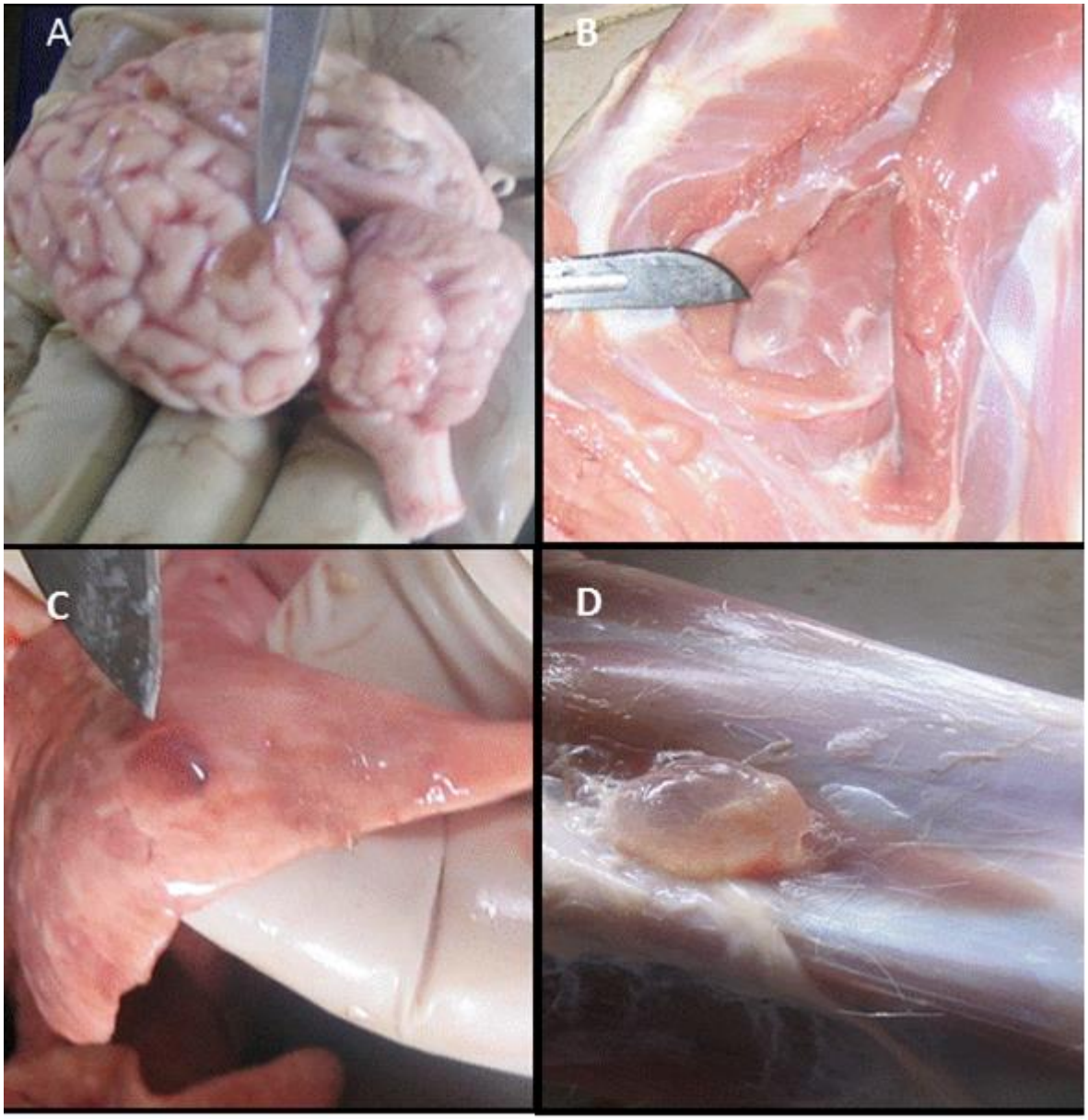

Figure 7 - Experimental infected goat with *Taenia multiceps* cyst (A and B) two and half months after infection (C and D) five and half months post-infection

4.2. Molecular characterization of *Taenia multiceps* from Mozambique

4.2.1. Polymerase chain reaction

Amplified products from *T. multiceps* were 450 bp and 550 bp for COI and ND1 subunits (**Figure 9**).

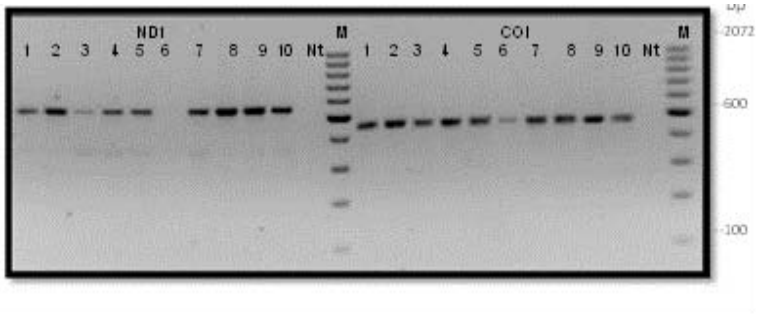

Figure 8 - PCR ND1 and COI amplified product for analysed cysts (from lane 1 to 9), positive control (lane 10) and negative control (NT).

1 – 15C, 2 – 9M, 3- 19M, 4 – 28M, 5- 35M, 6 – 34M, 7 – 7C, 8 -10C, 9 – 16M, 10 – positive control, Nt – Negative.

4.2.2. Sequence

Sequencing of the mitochondrial Cytochrome *c* oxidase subunit I (COI), and NADH dehydrogenase subunit 1 (**ND1**) revealed that the 14 cysts from goats in Tete abattoir belong to metacestodes of *T. multiceps*.

4.2.3. Phylogenetic tree

From phylogenetic tree constructed using individual sequences of sampled and data set, for the two subunit (NDI and CO1) it could bee see that all sequenced samples belongs to *Taenia multiceps*. On **Figure 11** and **12** phylogenetic trees are represented. Similar topology, with some differences, could be observed on two constructed trees. For both trees *T. multiceps* was most similar genetically to *T. seerialis* and *T. madoque*, although for CO1 *T. saginata* showed to have greater similarities. Others analysed Taenia sequences; *T. solium*, *T. cracisseps*, *T. hydatigena*, *T. regis*, *T. pisiformis* and *T. taenuiformis*.

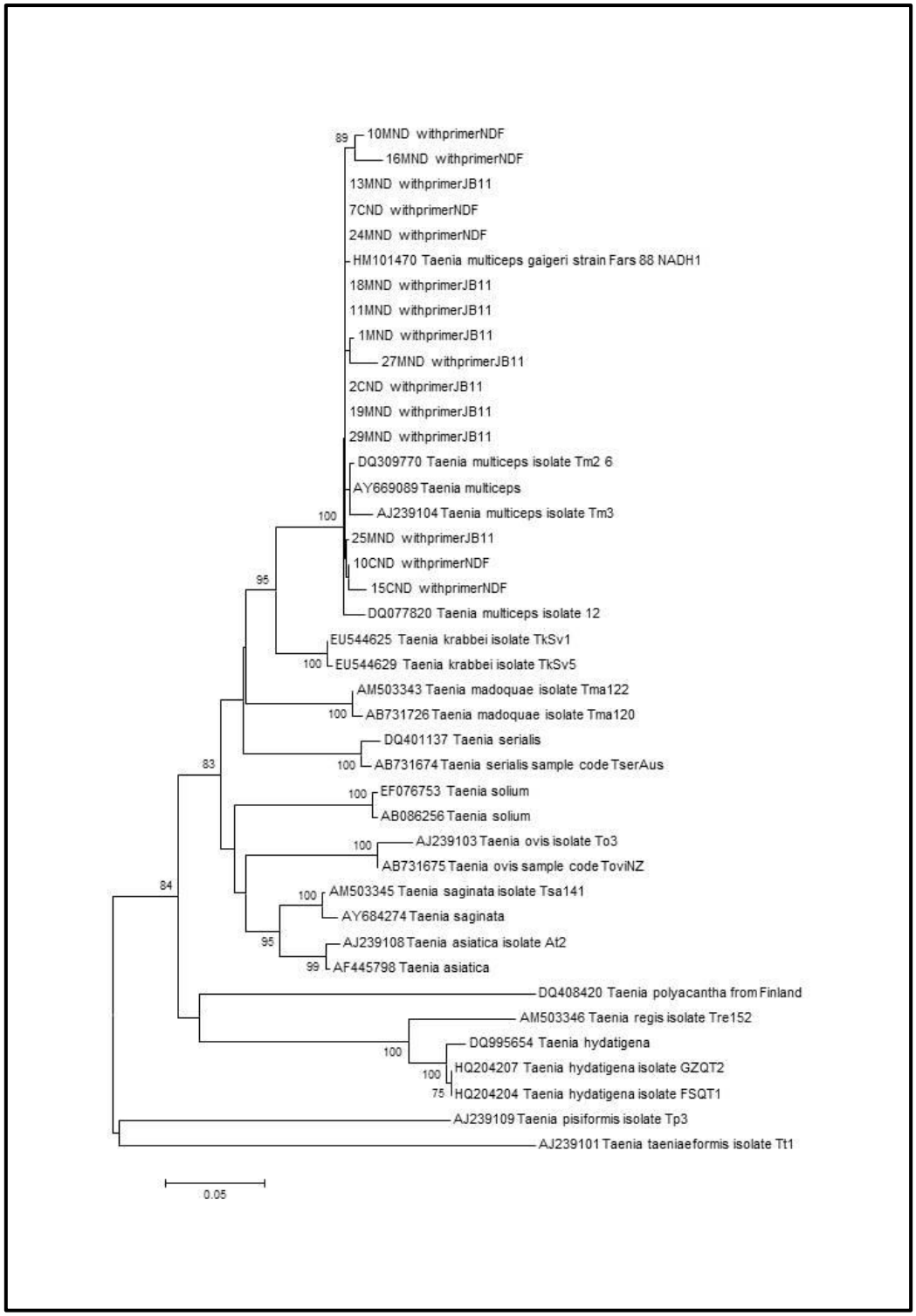

Figure 9 - Phylogenetic tree obtained for *Taenia multiceps* from Tete, Mozambique and others *Taenia spp*. from GenBank ND1

Taenia multiceps muscular cysts (1MND, 10MND, 11MND, 13MND, 16MND, 18MND, 19MND, 24MND, 25MND, 27MND and 29MND) and cerebral cysts (2CND, 7CND, 10CND and 15CND).

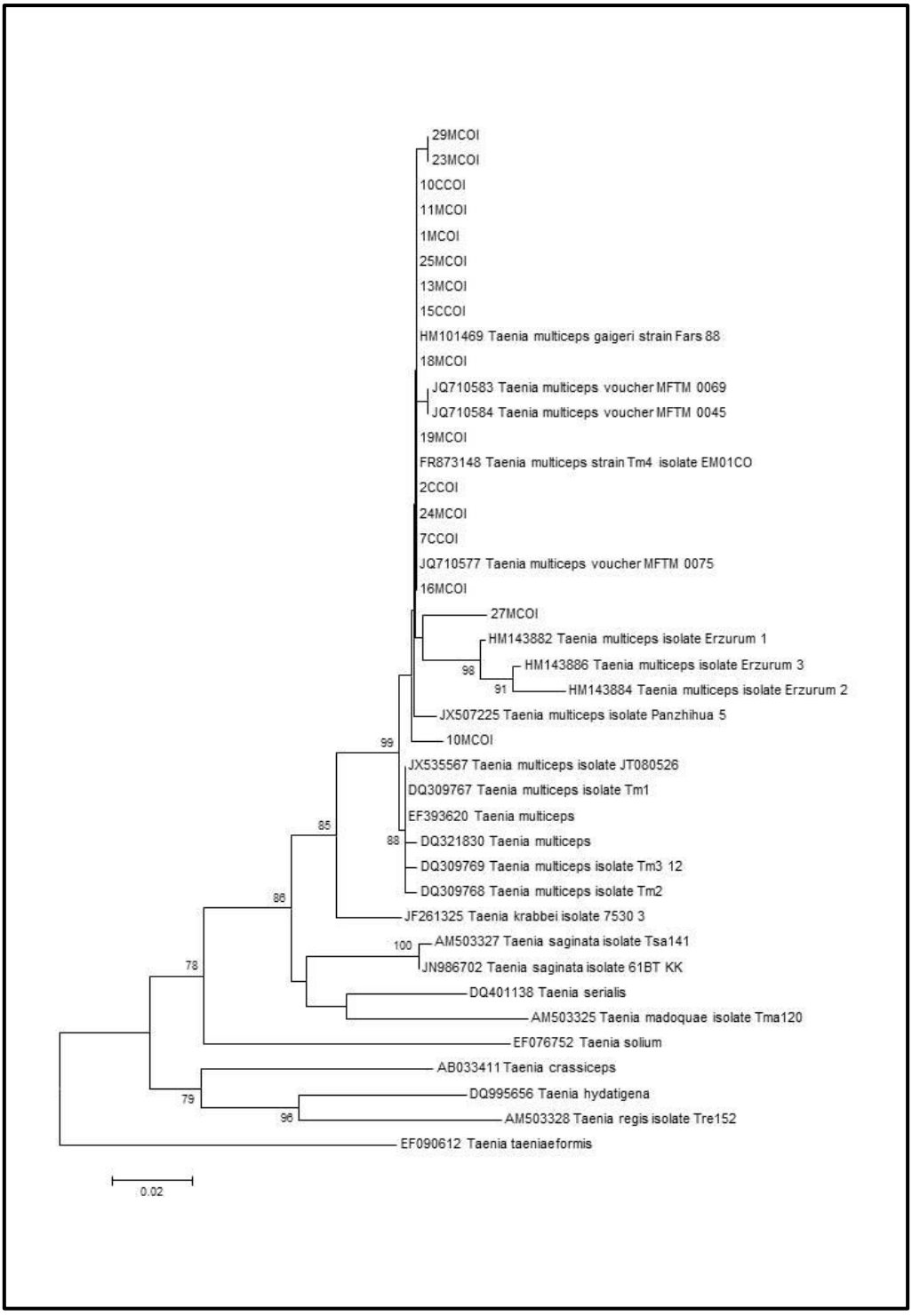

Figure 10 - Phylogenetic tree obtained for *Taenia multiceps* from Tete, Mozambique and others *Taenia spp*. from GenBank COI.

Taenia multiceps muscular cysts (1MCOI, 10MCOI, 11MCOI, 13MCOI, 16MCOI, 18MCOI, 19MCOI, 24MCOI, 25MCOI, 27MCOI and 29MCOI) and cerebral cysts (2CCOI, 7CCOI, 10CCOI and 15CCOI).

4.3. Antigenic characterization of *Taenia multiceps*

4.3.1. Antigen Protein quantification

Protein concentrations were higher for AgFE (23.85 μg/ml), Ts (10.31μg/ml) and Th (10.612μg/ml) for ultraviolet absorption, BCA and EZQ assays respectively. The lowest protein concentration for the three assays was obtained for AgO. Protein concentrations for the three performed assays are presented in **Table 10**.

Table 10- Protein Concentration (µg/ml) determined by ultraviolet absorption, BCA and EZQ.

Antigen	Protein concentration (μg/ml)		
	Ultraviolet absorption	BCA	EZQ
Taenia solium (fluid+membrane) (Ts)	ND	10.31	ND
Taenia hydatigena (fluid + membrane) (Th)	ND	6.83	10.612
Taenia multiceps (oncosphere) (AgO)	0.210	0.444	0.206
Taenia multiceps (fluid+membrane)(AgFE)	23.85	7.71	7.628
Taenia multiceps (fluid) (AgF)	2.6	2.8	0.3

BCA - Bicinchoninic acid for the colorimetric detection and quantification of total protein, EZQ - protein quantification kit EZQ (Invitrogen®) a fluorescence-based test, ND - not done.

4.3.2. SDS-PAGE western blot

Electrophoretic analysis of the antigen from *T. multiceps* (AgF, AgFE and AgO), *T. solium* (Ts) and T. *hydatigena* (Th) metacestodes revealed different protein band patterns (Figure 12). Higher numbers of bands (11) were detected in AgFe and Th while for AgO only two protein bands were observed. The molecular weights of the detected protein bands ranged from 160KDa to 10 KDa, most of the protein components were located in the range between 90 KD and 36.5 KDa. The pattern of protein distribution of AgFe and Th were similar, although there were minor differences on bands thickness.

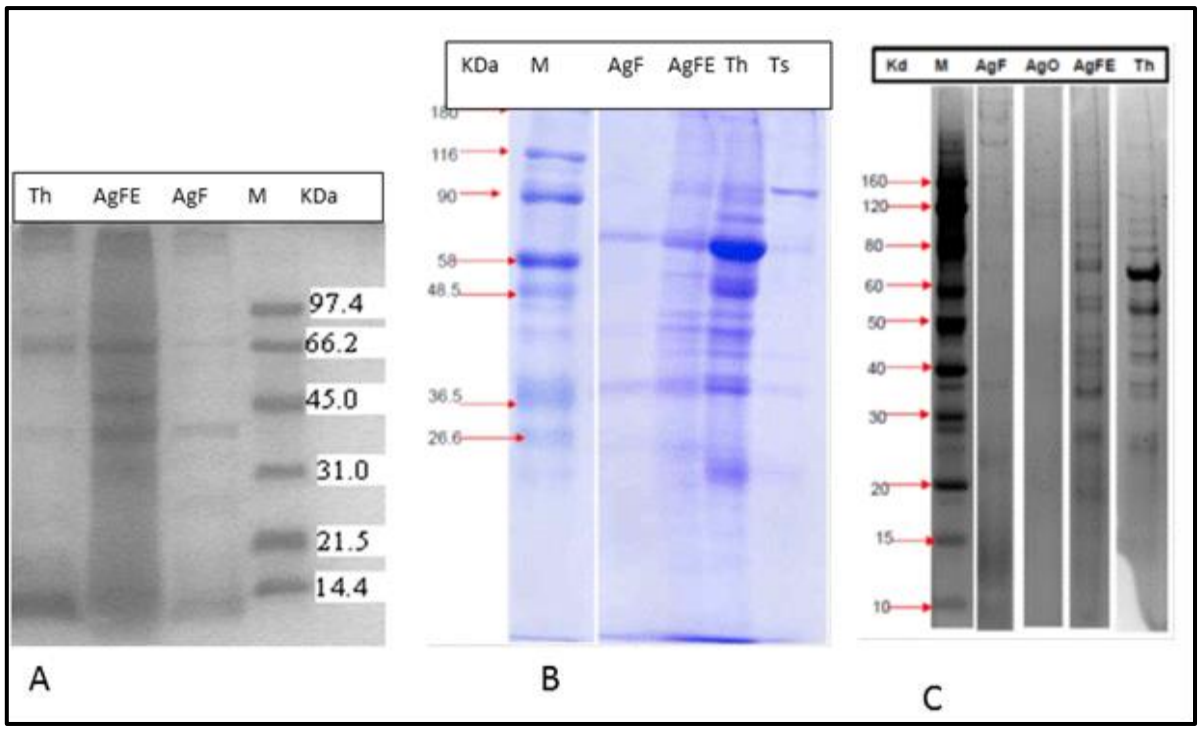

Figure 11 - Detected protein bands in crude antigen of *Taenia hydatigena* fluid and membrane (Th), *Taenia multiceps* onconphere (AgO), *Taenia multiceps* fluid (AgF), *Taenia multiceps* fluid and membrane (AgFE) and *Taenia solium* fluid and membrane (Ts).

Stained with comasie blue A and B and chemiluminuscence C.

On western blot, on experimental goat with 16 *T. multiceps* cysts, using 3,3' - diaminobenzidine (DAB, Sigma-Aldrich®) as substrate revealed four weak bands on the strips (Figure 13). One band, slightly above 36.5 KDa was observed in negative and positive goat sera. Protein bands below 26.5KDa were observed 30 days after infection. The bands located between 36.6 to 48.5 KDa and from 58 to 116 KDa were observed 45 days after infection. Western blot results using chemiluminescent in eight goats, 4 negatives and 4 experimental infected are presented in Figure 14. Due to low amount of protein in AgO no western blot were performed with this antigen. In all sera a strong protein band were detected between 80 and 60 KDa in all used antigens Th, AgF and AgFE. Reaction with AgF also showed in negative and positive sera two bands above 60 KDa and between 60 to 50KDa. One hundred and sixty five days after experimental infection all goats presented bands between 40 and 15 KDa, a very dark band was observed in goat with the highest number of cysts with the AgF.

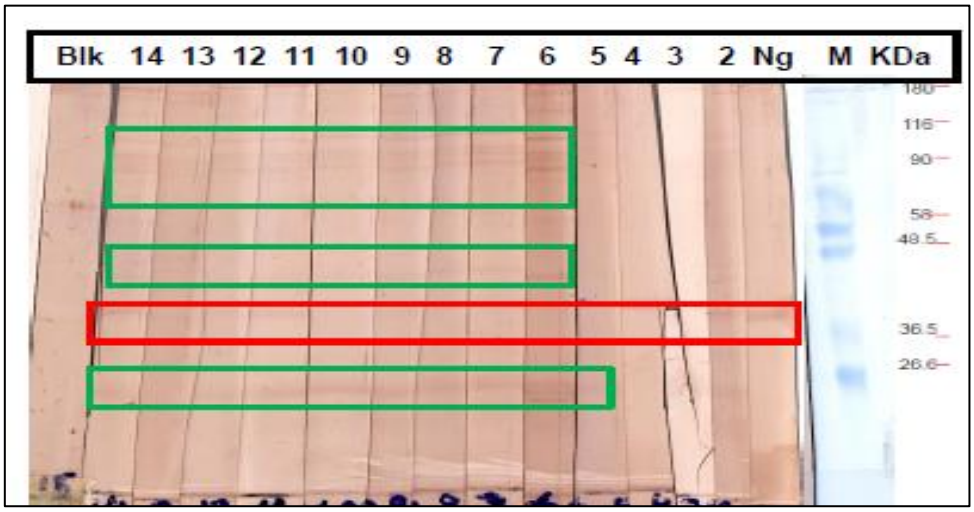

Figure 12 - Western blot detected bands using 3'3 -Diaminobenzidine in experimental infected goat.

Blk: Blank, Ng: Negative, 2: Natural negative goat, 3: Before infection, 4: 15 days after infection, 5: 30 days after infection, 6: 45 days after infection, 7: 60 days after infection, 8: 75 days after infection, 9: 90 days after infection, 10: 105 days after infection, 11: 120 days after infection, 12: 135 days after infection, 13: 150 days after infection, 14: 165 days after infection.

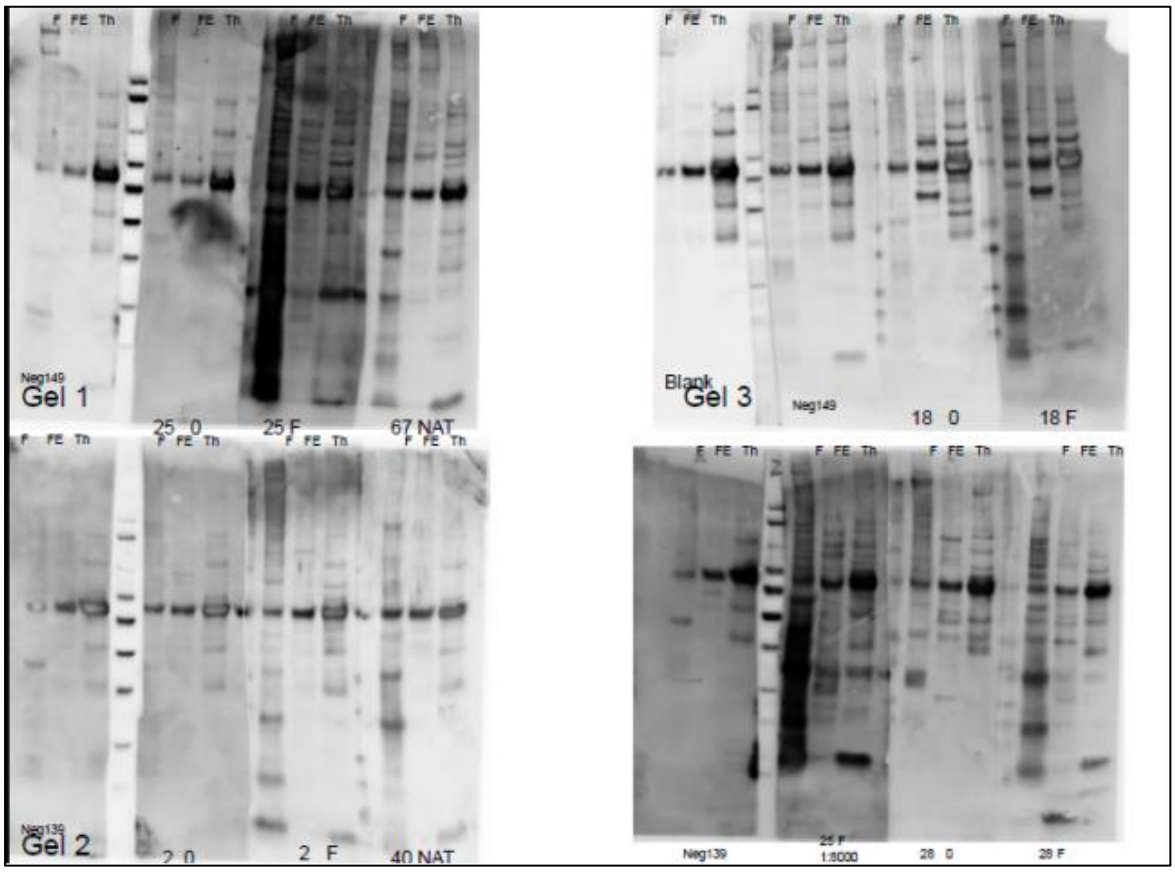

Figure 13 - Western blot detected bands using chemiluminescent.

Blk: Blank, Ng: Negative, 149 and 139: negative goat from non-endemic area, 67NAT and 40 NAT: Negative goats from endemic area, 25, 18, 2 and 28: experimental infected goats, 0: Before infection, F: 165 days after infection.

4.3.3. Antibody and antigen detected by ELISA

From 16 *T. multiceps* cysts non-infected goats, selected at the slaughterhouse, 5 (31.5%) and 4 (25%) were positive for antibodies (IgG) and antigen (HP10), respectively. From the five goats that tested positive for antibodies, 4 (80%) harboured *T. hydatigena* cysts. For the animals where the presence of HP10 antigen were detected no relation with a presence of other parasites could be established.

Five experimental infected goats, with cyst numbers ranging from 0-15, were tested for antibodies and circulating HP10 antigen. The tested sera included samples from 0 to 165 days after infection. **Table 11** presents the size, number and location of cysts in the five experimentally infected goats.

Table 11- The size, number and location of *Taenia* cysts in experimentally infected goats.

Animal Number	Size	Brain	Muscular and subcutaneous tissue	
	T. multiceps Cyst	*T. multiceps* Cyst	*T. multiceps* Cyst	*T. hydatigena*
Goat 1 (8)	4- 1.5	1	15	0
Goat 2 (10)	5-1.4	0	5	0
Goat 3 (23)	5 – 0.7	0	2	0
Goat 4 (103)	6.5- 1.5	0	4	2
Goat 5 (31)	0	0	0	0

Figure 15 presents the antibody and antigen profiles of five infected goats. In the antibody assay, positive results were observed 30 (goat 2 and 3), 45 (goat 1) and 75 (goat 5) days post infection. Goat 4 was positive in the antibody assay before and during the infection. In the antigen (Hp10) capture assay in 3 (goat 1, 2 and 3) positive animals were observed from 15 to 150 days after infection. Similarly to the results of the antibody assay, goat 4 presented a positive reaction in the antigen assay before infection until 45 days after infection. Goat five, who had no cysts after the experimental infection, was negative in the antigen assay, during all study period and a positive in the antibody assay from 75 to 120 days post infection.

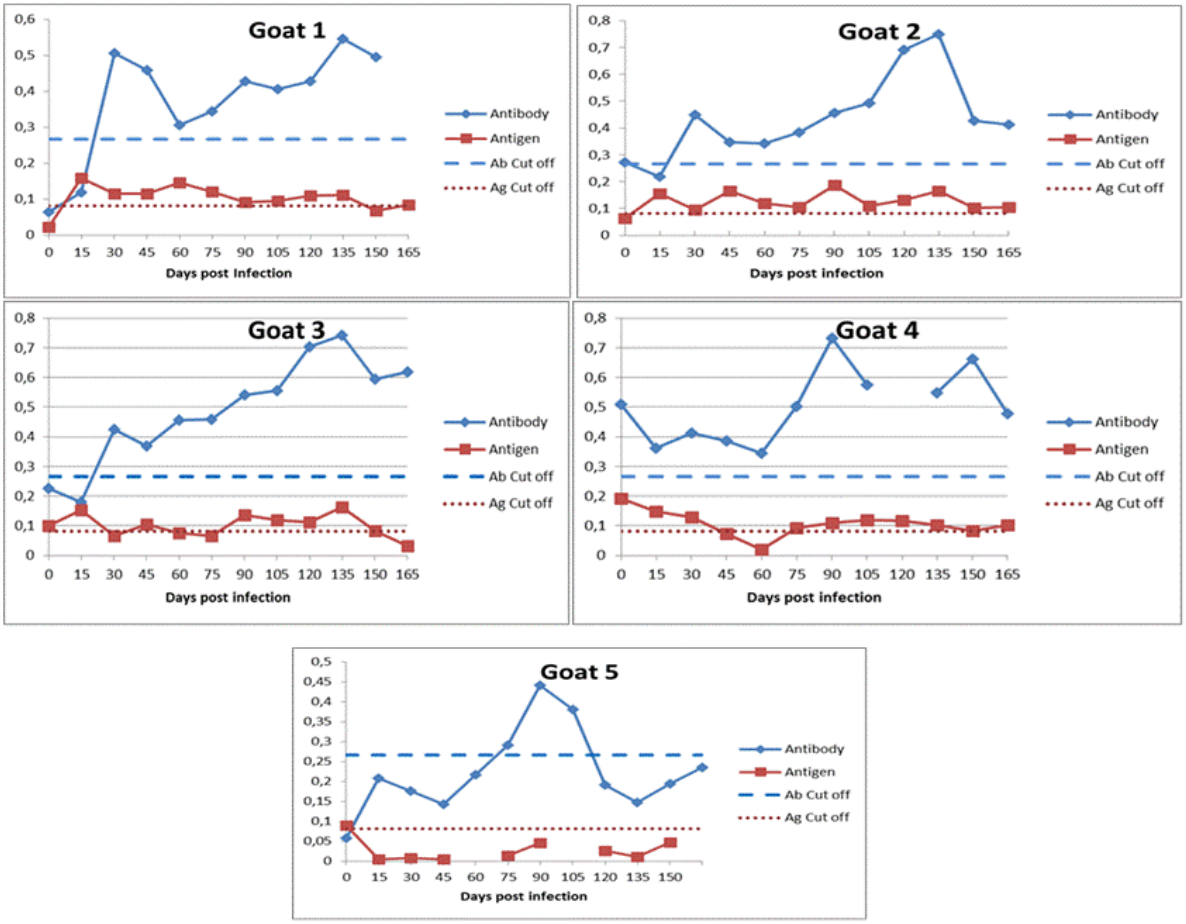

Figure 14 - Antigenic and antibody profile of five experimental infected goats.

4.4. Pharmacokinetic of albendazole in goats

After oral administration, ABZ was not detected in plasma, only the metabolites ABZSO and $ABZSO_2$ were observed. Using the described chromatographic condition ABZSO, $ABZSO_2$ and OXB were well resolved without interference from endogenous compounds with retention times of 2.933±0.222, 3.457±0.175 and 4.879±0.186 min respectively. ABZ did not appear in detectable amounts in plasma at any time.

4.4.1. Pharmacokinetics in healthy animals

Following a single oral administration dose of ABZ 10 mg/kg, ABZSO and $ABZSO_2$ were detected in plasma. The mean plasma concentration profiles and the standard deviation of ABZ metabolite (ABZSO and $ABZSO_2$) found in the study groups are represented in Figure 16.

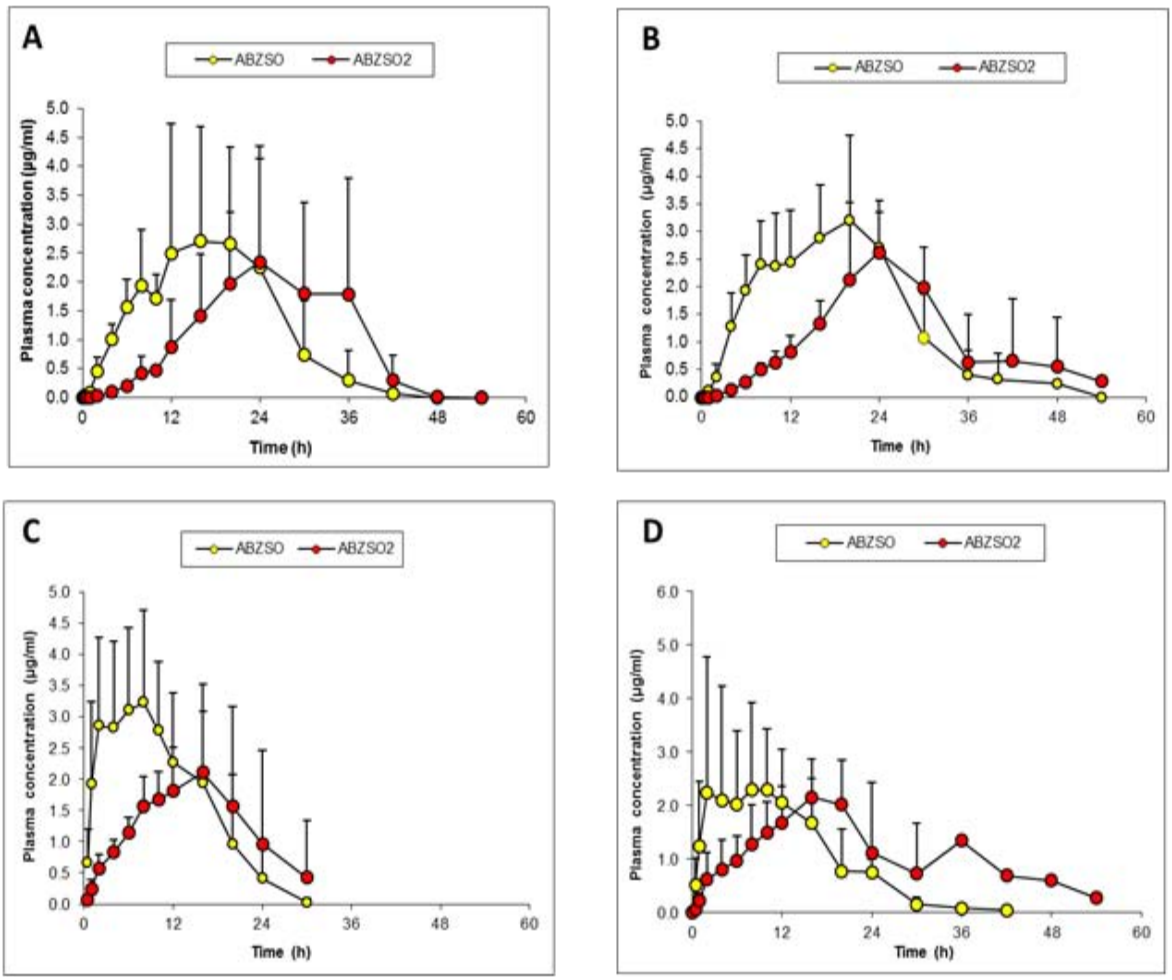

Figure 15 - Mean (±SD) plasma concentration time curve (n=6) of ABZSO and $ABZSO_2$ obtained after administration of 10 mg/kg ABZ to (A) adult female (B) young female (C) adult male (D) young male goats.

Plasma concentrations of ABZSO and $ABZSO_2$ in both groups of females (adults and young) were similar profile. In females, higher plasmatic concentrations of ABZSO were observed between 12 to 24 h, while $ABZSO_2$ was observed later, between 20- 30 h. Plasma concentrations of ABZSO in adult males were higher than those of young males maximum concentrations were observed between 4 - 8 h after the administration of ABZ and in young animals between 4 and 12 h. In males, plasma concentrations of $ABZSO_2$ were similar in both age groups. Earlier and higher plasmatic concentrations of ABZSO was found in adult males than in adult females, but no significant differences were observed. In young animals, maximum plasma concentrations for both metabolites were similar but appeared earlier in males than in females.

Table 12 - Pharmacokinetic parameters (Mean±SD) of ABZSO and $ABZSO_2$ obtained after treatment with oral doses 10mg/kg ABZ to healthy goats (adult and young, male and female)

		Cmax (μg/ml) Mean± SD	Tmax (h) Mean± SD	AUC_{0-t} (μg.h/ml) Mean± SD	MRT (h) Mean± SD
ABZSO	Adult female goat	3.03±2.20	16.67±4.68[a]	59.92±41.46	16.58±2.06[a]
	Young female goat	3.08±1.33	4.50±4.42[a]	42.20±19.80	7.57±2.13[a]
	Adult male goat	4.40±2.06[a]	18.33±5.99[a]	75.72±27.07[a]	17.98±2.08[a]
	Young male goat	2.98±2.20[a]	6.50±3.99[a]	31.28±34.93[a]	8.87±2.67[a]
$ABZSO_2$	Adult female goat	2.50±1.83	23.33±1.63[b]	47.38±42.64	22.80±3.42[b]
	Young female goat	2.60±1.13	12.00±3.35[b]	33.72±18.77	10.87±2.96[b]
	Adult male goat	3.67±1.62	25.33±3.39[b]	59.67±26.43	24.38±3.66[b]
	Young male goat	2.48±1.49	13.00±4.34[b]	30.38±18.05	14.10±6.00[b]

a. Significant difference between ages in the same sex at $p<0.05$ for ABZSO
b. Significant difference between ages in the same sex at $p<0.05$ for $ABZSO_2$

In **Table 12** the mean of pharmacokinetic parameters of ABZSO and $ABZSO_2$ is detailed for the different studied goat groups. For both metabolites, ABZSO and $ABZSO_2$, the mean values of Tmax and MRT are significantly higher in adult females (Tmax=16.67±4.68 h and MRT=16.58±2.06 h) than in young females (Tmax=4.50±4.42 and MRT=7.57±2.13 h). The Cmax and AUC values for ABZSO and $ABZSO_2$ are similar in adult and young females.

ABZSO presented a higher Cmax (4.40±2.06 μg/ml) in adult males than in young males (2.98±2.20 μg/ml), but no statistical significant differences were observed. A significant statistical difference between male adult and young groups was observed for Tmax, AUC and MRT values for ABZSO. A significant statistical difference between female adult and young groups was observed for Tmax and MRT values for ABZSO.

$ABZSO_2$ appeared in plasma more slowly than ABZSO. ABZSO *Cmax* (3.67±1.62 and 2.48±1.49 μg/ml, respectively) and AUC (59.67±26.43 and 30.38±18.05 μg.h/ml, respectively) were higher in adult males than young males, but non-significant statistical differences between groups were observed. For *Tmax* and MRT of $ABZSO_2$ significant statistical differences were observed.

4.4.2. Pharmacokinetics in males infected with *Taenia multiceps*

4.4.2.1. Pharmacokinetics

Following administration of 10 and 20 mg/kg of ABZ in adult male goats experimentally infected with *T. multiceps*, the kinetic profiles of ABZSO and $ABZSO_2$ were studied.

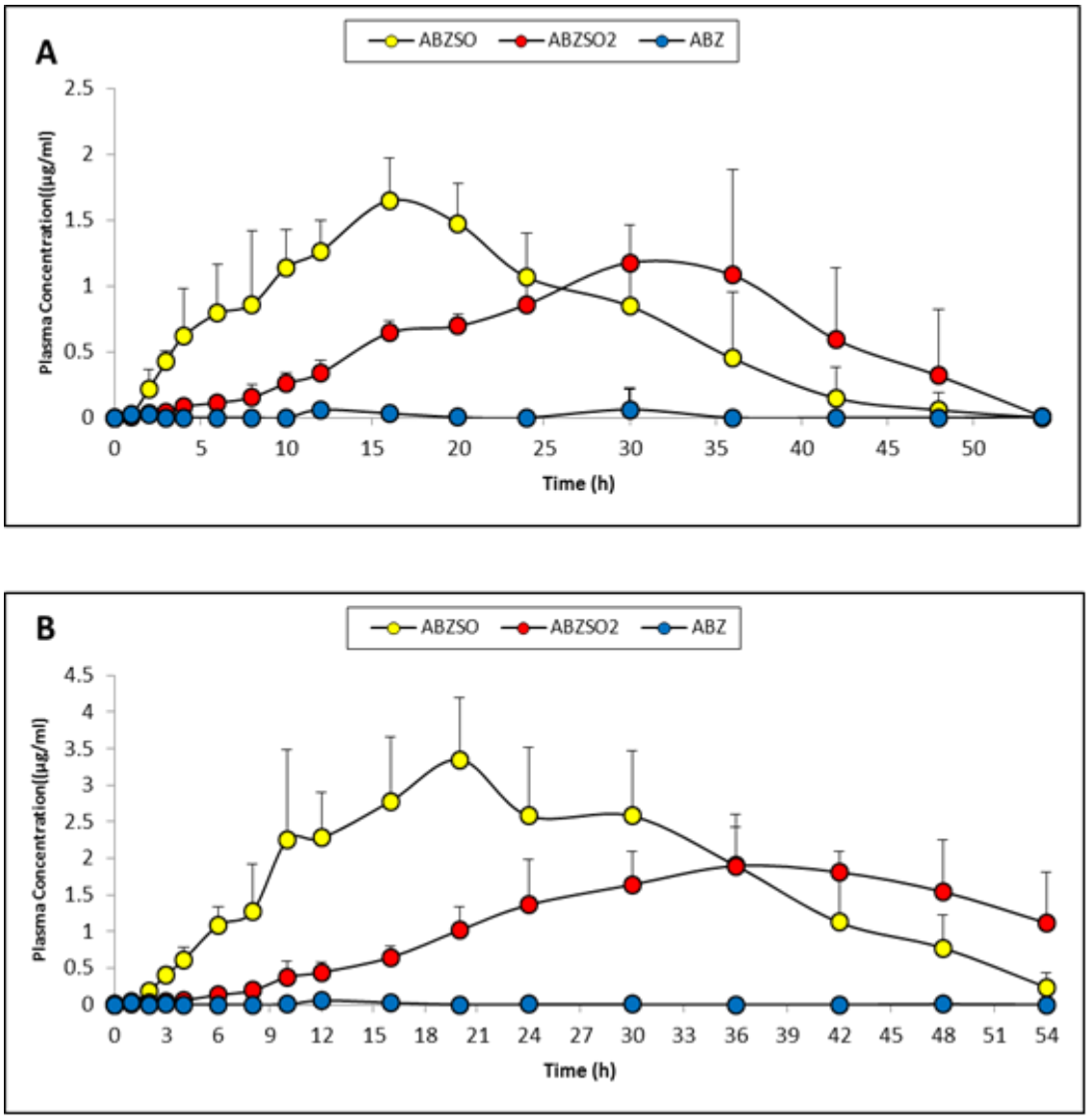

Figure 16 - Mean (±SD) plasma concentration time curve (n=7) of ABZSO, $ABZSO_2$ and ABZ obtained after oral administration (A) 10mg/kg (B) 20mg/kg from infected adult male goats.

Figure 17 shows that ABZ is present in plasma at very low concentrations. ABZSO and $ABZSO_2$, appeared at substantial amounts in plasma. In both doses, ABZSO were higher than $ABZSO_2$ in the first hours. After a dose of 20 mg/kg, plasma concentrations of ABZSO and $ABZSO_2$ were higher, and remained in plasma for a longer time (more than 10 hours) than

after the administration of 10 mg/kg dose. The plasma concentration profiles of ABZSO, $ABZSO_2$ and ABZ in 10 and 20 mg/kg were similar for the two different doses.

With the kinetic analysis of plasma concentration curves of ABZSO and $ABZSO_2$ represented in **Figure 17** the pharmacokinetic parameters of each curve were obtained. The parameters are shown in **Table 13 and 14**.

Table 13 - Individual pharmacokinetic parameters for ABZSO and $ABZSO_2$ after oral administration of 10 mg/kg of ABZ to adult male goats infected with *Taenia multiceps.*

		Cmax (µg/ml)	**Tmax (h)**	**AUC^{0-t} (µg.h/ml)**	**MRT (h)**	**T1/2 (h)**
ABZSO	A	1.9	20	32.6	19.4	2.7
	B	1.3	12	28.6	18.1	2
	C	2	16	44.9	19	2.8
	D	1.6	12	36.4	17	2.5
	E	1.7	16	48.3	23.6	2.5
	F	1.7	16	31.8	14.9	1.8
	G	1.6	20	38.5	12.8	7.4
	Mean (±SD)	1.7± 0.2	16±3.3	37.3±7.2	19.5±3.5	2.4±0.4
$ABZSO_2$	A	1	36	23.4	29.5	2.5
	B	1.5	30	25.8	26	3.7
	C	1.4	30	36.5	29.3	1.7
	D	1.3	30	27.5	25.2	2.3
	E	2.5	36	47	31.4	1.6
	F	0.8	30	18.2	23.6	5.7
	G	1.4	42	35	36	3,1
	Mean (±SD)	1.4±0.5	33.4±4.7	30.5±9.7	28.7±4.3	2.9±1.6

Table 14 - Individual pharmacokinetic parameters for ABZSO and $ABZSO_2$ after oral administration of 20 mg/kg of ABZ to adult male goats infected with *Taenia multiceps.*

		Cmax (μg/ml)	Tmax (h)	AUC^{0-t} (μg.h/ml)	MRT (h)	T1/2 (h)
ABZSO	A	2.8	24	71.4	21.8	3.1
	B	4.0	30	117.1	26.3	3.1
	C	3.0	24	90.9	26.7	3.1
	D	2.7	20	98	32.4	9.0
	E	4.8	10	82.8	24.6	2.3
	F	3.0	24	98.3	27.8	2.4
	G	4.3	20	119.9	24.2	4.0
	Mean (±SD)	3.5±0.8	21.7±5.7	96.9±16.1	26.6±3.1	3.86±2.2
$ABZSO_2$	A	2.8	36	71.3	30	1.5
	B	2.3	30	71.7	40	8.5
	C	2.0	48	59.3	48	12.6
	D	1.8	54	55.8	37.8	3.1
	E	1.9	42	47.2	33.5	2.9
	F	2.0	36	48.9	44.7	11.5
	G	1.9	48	46.3	72.5	29.3*
	Mean (±SD)	2.1±0.3	42±7.9	57.2±10.0	43.8±13.0	7.7±4.5

*Not included in mean

Individual pharmacokinetic parameters of ABZSO and $ABZSO_2$ are presented in **Table 13 and 14**. Maximum plasma concentration for ABZSO after administration of 10 and 20 mg/kg doses, were 1.7± 0.2 μg/ml and 3.5±0.8 μg/ml, respectively and these values were obtained after 16±3.3 h and 21.7±5.7 h. respectively. In both doses (10 and 20 mg/kg), Tmax values for ABZSO were lower than $ABZSO_2$. In the 10 mg/kg dose values were 16±3.3 h and 33.4±4.7 h, respectively, and in the 20 mg/kg dose values were 21.7±5.7 h and 42±7.9 h, respectively. The individual AUC of ABZSO and $ABZSO_2$ reached 37.3±7.2 (μg.h/ml) and 29.8±9.5 (μg.h/ml) respectively, after administration of the 10 mg/kg dose and 96.9±16.1 and 57.2±10.0, respectively for the 20 mg/kg dose. After administration of 10 or 20 mg/kg doses a Cmax, Tmax, AUC and MRT were obtained with differences statistically significant between both doses. The T1/2, the elimination terminal phases was similar between both doses.

Table 15 - Statistical analysis of albendazole sulphoxide and albendazole sulphona pharmacokinetic parameters obtained after administration of a ingle doses of 10 mg/kg of ABZ to infected or healthy goats, and 10 or 20 mg/kg to infected goats.

ABZSO	Cmax (μg/ml)	Tmax (h)	AUC $^{0-90}$ (μg.h/ml)	MRT (h)	T1/2 (h)
Healthy male 10mg/kg	4.4±2.1[a]	18.3±5.9	75.7±27.1[a]	17.9±2.1	3.2±2.2
T. multiceps 10mg/kg	1.7± 0.2[a,b]	16.0±3.3[b]	37.3±7.2[a,b]	19.5±3.5[b]	2.4±0.4
T. multiceps 20mg/kg	3.5±0.8[b]	21.7±5.7[b]	96.9±16.1[b]	26.6±3.1[b]	3.9±2.2
$ABZSO_2$					
Healthy male 10 mg/kg	3.7±1.6[a]	25.3±3.4[a]	59.7±26.4[a]	24.4±4.3	4.3±4.2
T. multiceps 10 mg/kg	1.4±0.5[a,b]	33.4±4.7[a,b]	30.5±9.7[a,b]	28.7±4.3[b]	2.9±1.6
T. multiceps 20 mg/kg	2.1±0.3[b,]	42.0±7.9[b]	57.2±10.0[b]	43.8±13.0[b,]	11.1±9.1

[a]indicates significant differences between group healthy male treated with 10 mg/kg of ABZ and infected male treated with 10mg/kg of ABZ ($p<0.05$)
[b]indicates significant differences between two infected groups treated with 10 or 20 mg/kg of ABZ ($p<0.05$)

Results from statistical analysis of pharmacokinetic parameters after administrations of single doses of 10 mg/kg of ABZ to healthy or infected goats, are presented in **Table 16.** Cmax and AUC for both metabolites, obtained after drug administration a single dose, were statistically significantly ($p<0.05$) lower in *T. multiceps* infected goats (10 mg/kg) than in healthy goats. Tmax, MRT and T1/2 did not show statistically significant differences between groups.

When pharmacokinetic parameters of ABZSO and ABZSO are compared, from infected goats treated with 10 or 20 mg/kg of ABZ, it can be seen that there are statistically significant differences in the pharmacokinetic parameters (Cmax, Tmax, AUC and MRT) between healthy and infected goats. No differences were observed for T1/2.

Kinetic profiles of ABZSO and $ABZSO_2$ were studied after an oral dose of 10mg/kg administered during 3 consecutive days (every 24 h) to adult male goats (n=7) infected with *T. multiceps*.

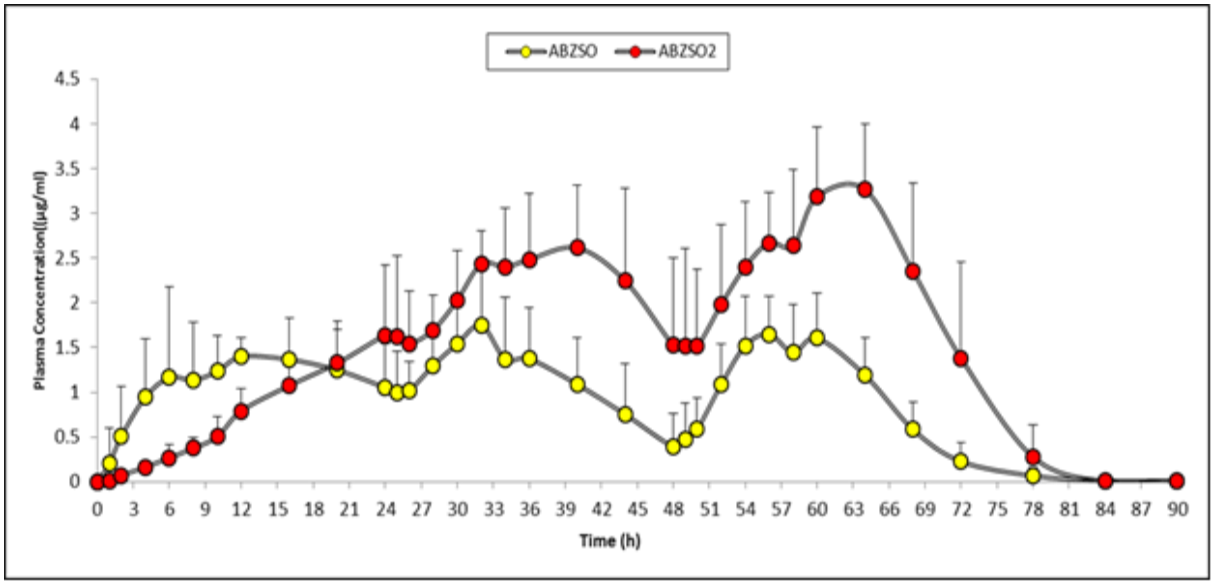

Figure 17 -Disposition curve of plasma albendazole metabolites (ABZSO and $ABZSO_2$) in male goats (n=7) after a 3 repeated doses of albendazole at 10 mg/kg.

The plasma concentration curve of ABZSO and $ABZSO_2$ obtained after ABZ oral administration dose of 10 mg/kg, applied every 24h during 3 consecutive days, to infected goats are shown in **Figure 17**. After the first administration, the concentration of ABZSO was higher than the $ABZSO_2$ but after the second and third administrations, $ABZSO_2$ plasma concentrations increased and were higher than those observed for ABZSO. For ABZSO, the high plasma concentrations were observed around 9-12 h, 30-36 h and 54-63 h after first, second or third administration, respectively. Maximum plasma concentration for $ABZSO_2$ were observed slightly later, 18-24 h, 33-45 h and 60-66 h after the first, second or third administration, respectively. After 60 h ABZSO and $ABZSO_2$ plasma concentrations decrease, and you can see a rapid elimination phase.

Using the plasma concentrations of multiple administration of ABZ at a dose of 10m/kg, the pharmacokinetic parameters were calculated for each infected goat under study.

Table 16 - Individual pharmacokinetic values ABZSO after oral administration of ABZ with a multiple doses of 10 mg/kg for 3 consecutive days, to adult male goats (n=7) infected with *Taenia multiceps*

	Cmax µg/ml			Tmax H			AUC_{0-t} µg.h/ml		
Time	0/24h	24/48	48/72h	0/24	24/48 Rt (Pat)	48/72 Rt (Pat)	0/24	24/48	48/72
Animal									
A	1.5	1.1	1.3	12	30 (6)	64(16)	24.5	17.6	19.5
B	1.6	1.8	1.9	20	32 (8)	56 (8)	23.6	31.4	26.4
C	2.0	1.9	1.6	10	32 (8)	60(12)	36.1	33.8	27.8
D	1.9	1.7	2.5	20	32 (8)	58(10)	25.9	35.4	39.6
E	1.2	0.9	1.2	16	30 (6)	56 (8)	20.4	11.9	17.7
G	2.1	2.5	1.5	16	32 (8)	56 (8)	34.1	35.6	21.1
H	1.1	2.4	2.3	8	32 (8)	54 (6)	22.3	25.0	25.1
Mean±SD	**1.9±0.5**	**1.8±0.6**	**1.8±0.5**	**13.7±6**	**7.4±3**	**9.7±3.4**	**26.7±6.0**	**27.2±9.4**	**25.3±7.3**

Rt –Real time, Pat – Partial time.

Table 17 - Individual pharmacokinetic values $ABZSO_2$ after oral administration of ABZ with a multiple doses of 10 mg/kg for 3 consecutive days, to adult male goats (n=7) infected with *Taenia multiceps*

	Cmax µg/ml			Tmax h			AUC_{0-t} µg.h/ml		
Time	0/24	24/48	48/72	0/24	24/48 Rt (Pat)	48/72 Rt (Pat)	0/24	24/48	48/72
Animal									
A	1.4	2	3.3	24	36(12)	64(16)	23.9	37.5	45.8
B	1.4	2.9	3.1	24	44(20)	58(10)	13.4	52.9	53.3
C	1.9	2.9	3.7	24	44(20)	64(16)	17.3	56.5	67.5
D	2.2	3.9	4.5	24	44(20)	64(16)	19.7	67.2	89.5
E	2.2	2.5	3.4	24	32(8)	64(16)	19	42.8	61.3
G	2.2	3.9	3.1	24	36(12)	64(16)	24.7	67.7	50.7
H	1.2	2.7	3.6	16	40(16)	60(12)	15.6	38.6	42.9
Mean±SD	**1.8±0.5**	**2,9±0.7**	**3.5±0.5**	**22.9±3[a]**	**15.4±4.9[a]**	**14.6±2.5[a]**	**18.0±3.6[a]**	**51.9±12.7[a]**	**58.7±16.0[a]**

Rt –Real time, Pat – Partial time. [a]Sginificant differences between administrations

In **Tables 16** and **17,** individual pharmacokinetic parameters for ABZSO and $ABZSO_2$ are presented. ABZSO Cmax and AUC were similar in each one of the time intervals between the three doses administered. For $ABZSO_2$, Cmax and AUC increased with the time during the three administered doses. Tmax values for $ABZSO_2$ are significantly ($p<0.05$) higher than Tmax values for ABZSO in the three administration periods. When the Tmax are compared for both metabolites, it can be observed that these times are significantly reduced ($p < 0.05$)

from first administration to the second but no significant variation ($p > 0.05$) appears from the second to third administration.

Table 18 - Statistical analysis of albendazole sulphoxide and albendazole sulphona pharmacokinetic parameters obtained after administration of a single doses of 10 and 20 mg/kg to infected and healthy goats and 10 mg/kg/3 days

ABZSO	Cmax (μg/ml)	Tmax (h)	AUC $^{0-24}$ (μg.h/ml)
Healthy male 10mg/kg	3.7±1.3[a,b]	17.3±6.4	53.8±17.3[b]
T. multiceps 10mg/kg	1.7±0.2[b]	16±3.0	23.3±4.5[b]
T. multiceps 20mg/kg	3.7±0.8[d]	19.7±4.8	42.6±7.6[d]
T. multiceps 10mg/kg/3days	1.9±0.5 [a,d]	13.7±6	26.7±6.0[d]
$ABZSO_2$			
Healthy male 10 mg/kg	2.8±1.2[b]	22±3.4	24.7±9.6[b]
T. multiceps 10 mg/kg	0.9±0.2[b,c]	22.9±1.8±	16.5±20[b,c]
T. multiceps 20 mg/kg	1.4±0.4	22.9±1.8	12.5±1.8[d]
T. multiceps 10mg/kg/3 days	1.8±0.5[c]	22.9±3	18.0±3.6[c,d]

[a]indicates significant differences between group healthy male treated with 10 mg/kg of ABZ and infected male treated with 10mg/kg/3 days of ABZ ($p<0.05$)
[b]indicates significant differences between healthy male treated with 10 mg/kg of ABZ and infected male treated with 10mg/kg of ABZ ($p<0.05$)
[c]indicates significant differences between group infected male treated with 10 mg/kg of ABZ and infected male treated with 10mg/kg/3 days of ABZ ($p<0.05$)
[d]indicates significant differences between group infected male treated with 20 mg/kg of ABZ and infected male treated with 10mg/kg/3 days of ABZ ($p<0.05$

When comparing the kinetic parameters of the interval (0 to 24h) after the first ABZ administration in the multiple dose treatment, with the same interval, and after the administration of single dose of ABZ to infected goats, statistically significant differences can be observed for $ABZSO_2$ Cmax and AUC. Nevertheless, no significant differences were observed for kinetics parameters of ABZSO (Table 18).

4.4.2.2. Presence of albendazole and its metabolites in tissues and cyst fluid

The presence of ABZ and its metabolites in plasma, brain, muscles and cyst fluid were studied in infected male goats (n=5), for an interval of 12 h after administration of an oral dose of 10 mg/kg of ABZ.

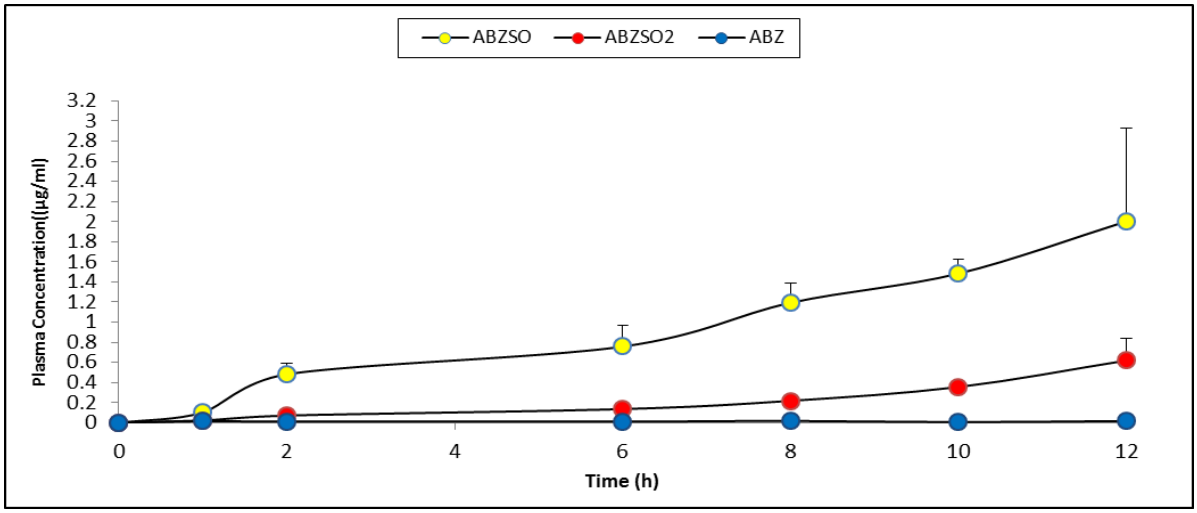

Figure 18 - Disposition curve of plasma ABZ, ABZSO and $ABZSO_2$ in experimental infected with *Taenia multiceps* male goats (n=5) after administration of oral dose of ABZ at 10 mg/kg. Concentrations observed during 12 hours after administration of ABZ.

The mean plasma concentrations of ABZ and its metabolites obtained from 0 to 12 hours are presented in Figure **18**. The metabolic profile was similar to that/those described in previous studies with the same dose. ABZSO showed higher values than $ABZSO_2$ and very little amount of ABZ was observed. Plasma concentrations of both metabolites gradually increased over time. 12 hours after the treatment the plasma concentrations were 2.0±0.9 μg/ml and 0.6±0.2 μg/ml for ABZSO and $ABZSO_2$, respectively.

Brain, muscles and fluid cyst were collected after animal sacrifice and analytical procedures were performed for detection of ABZ and their metabolites in these tissues.

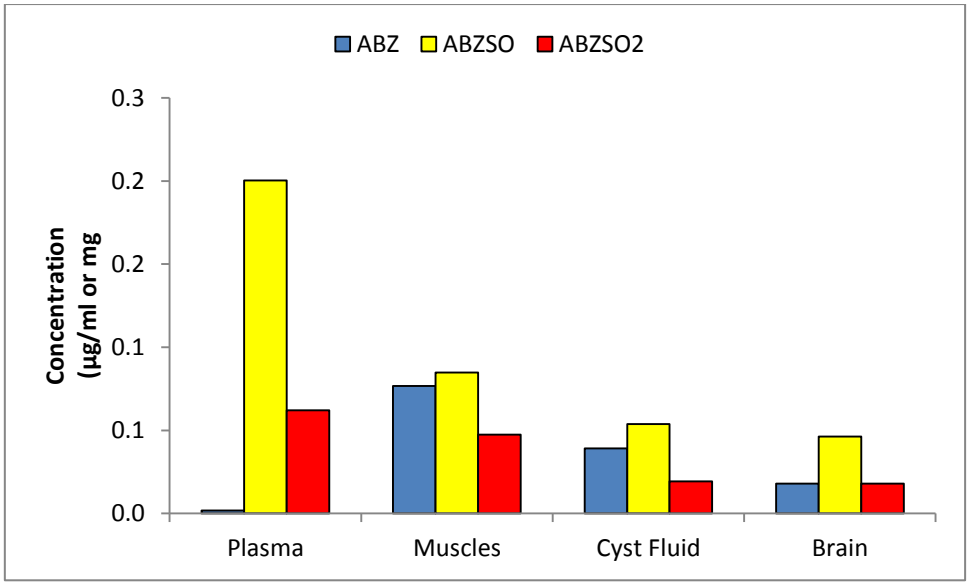

Figure 19 - Plasma, muscles, brain and cyst fluid concentrations of ABZ, ABZSO and $ABZSO_2$ 12 hours after oral doses of ABZ 10mg/kg in experimental infected male goats (n=5).

The amount of ABZ, ABZSO and $ABZSO_2$ detected in plasma, muscles, cyst fluid and brain, twelve hours after administration of an oral dose of ABZ of 10 mg/kg are presented in **Figure 19.** Twelve hours after drug administration, ABZ appears in higher concentrations in muscles followed by cyst fluid, brain and plasma, 0.77±0.53 μg/g, 0.40±0.008 μg/ml, 0.19±0.09 μg/g and 0.02±0.03 μg/ml respectively. Meanwhile, for the ABZSO and ABZSO2 metabolites, higher concentrations were found in plasma and lower in brain tissue. ABZSO always appeared in higher concentrations than $ABZSO_2$.

Individual amounts of ABZ, ABZSO and $ABZSO_2$ found in muscles, brain and cyst fluid in studied animals (n=5) are presented in **Table 19**.

Table 19 – Concentrations (µg/g or ml) of ABZ and its metabolites in muscles, brain, cyst fluid 12 hours after oral doses of ABZ 10mg/kg in experimental infected male goats (n=5).

	ABZ (µg/g or ml)			**ABZSO (µg/g or ml)**			**$ABZSO_2$ (µg/g or ml)**		
Animal	**Muscle**	**Cyst fluid**	**Brain**	**Muscle**	**Cyst fluid**	**Brain**	**Muscle**	**Cyst fluid**	**Brain**
A	0.41	0.05	0.11	1.55	0.38	0.12	0.62	0.18	0.12
B	0.37	0.04	0.09	0.97	0.57	0.09	0.36	0.17	0.09
C	1.47		0.13	0.23		0.13	0.43		0.13
D	1.23	0.04	0.24	0.09	0.66	0.24	0.49	0.24	0.24
E	0.36		0.32	1.40		0.32	0.45		0.32
Mean±SD	**0.7± 0.5**	**0.04±0.01**	**0.18±01**	**0.7± 0.5**	**0.04±0.01**	**0.18±01**	**0.7± 0.5**	**0.04±0.01**	**0.18±01**

High variability in concentrations of ABZ and its metabolites were observed in muscle. In muscle, two goats presented more than 1μg/g of ABZ and less amount of ABZSO than the other animals. Meanwhile, the quantity of $ABZSO_2$ was similar in all goats. Concentration of ABZ and its metabolites in cyst fluid and brain were almost the same.

Relative concentrations of ABZSO in tissues, compared with plasma concentrations were 43.6%, 17.2% and 11.3% for muscles, intra-cyst and brain respectively. And ABZ achieve a significant concentrations in muscle and cyst fluid.

4.5. Efficacy of albendazole against *Taenia multiceps* larvae in goats

From 57 infected animals, 41 were assessed for cyst viability and 11 animals were excluded due to non-establishment of infection and management constraints and 5 animals died before the evaluation. After exclusion of the 11 uninfected animals, the number of goats in each group was revised and the results are presented in Table 4. Recovered cysts from each group varied from 11 to 44 were found in different muscle and tissue with different size. In all treated groups, nonviable cysts were significantly higher ($P< 0.05$) than in the control group after two months post-infection. Efficacies for two months old cyst were 90.3%, 72.7% and 73.9% for group 1, 2 and 3, respectively (**Table 20**). In all cases, statistically differences ($P<0.05$) were observed between treated and control groups. In Table 1, it is possible to observe the efficacy in multiple drug administration but not in single administration.

Five months after infection, ABZ efficacy was 88.6% for multiple doses. At this age, mortality of *T. multiceps* larva was also observed in control group (**Table 20**).

Table 20 - Plasma, muscles, brain and cyst fluid concentrations of ABZ, ABZSO and $ABZSO_2$ 12 hours after oral doses of ABZ 10mg/kg in experimental infected male goats (n=5).

Group	Number goats	Time of treatment (months post infection)	Dosage regime	Total (Recovered cysts)	Total Non-viable cysts	% of non-viable cysts
G1	7	2	10 mg/kg/3 days	31	28	90.3[a]
G2	6	2	10 mg/kg	11	8	72.7[a]
G3	6	2	20 mg/kg	19	14	73.9[a]
G4	12	2	Control	44	6	13.6
G5	5	5	10 mg/kg/3 days	35	31	88.6
G6	5	5	Control	13	11	84.6

[a] Statistically Significant difference between treated and control group at $p<0.05$

DISCUSSION

5. Discussion

5.1. Baseline abattoir survey

In Mozambique, goats represent 95% of the national small ruminant population. A large proportion (97.6%) is raised by the smallholder sector providing an important source of income and wealth to the country (INE, 2011). Typically, goat herds are raised mainly on pasture for meat production. In this production system, infections by gastrointestinal parasites are considered a major cause of morbidity and mortality (Hoste et al., 2005; Rinaldi et al., 2007). Usually, gastrointestinal parasites are associated to infections with sub-clinical or chronic manifestations (Soulsby, 1982; Urquhart et al., 1996), which makes their early diagnosis particularly difficult. The combined effect of internal parasitic infections is regarded as an important constraint to animal production and productivity.

In this study, results of faecal examination revealed that all goats were positive for at least one parasite. Similar results were found in previous studies in goats from different tropical regions including 95.45% of infected animals in Cameroon (Mbuh et al., 2008), 93.29% to 95% in Ethiopia (Tafera et al., 2009), 92.4% in India (Bandyopadhyay et al., 2010), 88 to 97% in Zimbabwe (Pandey et al., 1994), 82% in Kenya (Maichomo et al., 2004) and 73.2 to 81.6% in Nigeria (Owhoeli et al., 2013). This high prevalence could be related to the management system that goats in Tete were subjected to, as they were always left to scavenge and feed indiscriminately, only kept in sheds at night. Additionally, there are no reports of veterinary interventions aiming at the treatment and control of parasitic infections in this region. In contrast, our results indicate a prevalence that is substantially higher than the, 53.19% prevalence reported in a similar survey conducted in an abattoir in Nigeria (Solomon-Wisdom et al., 2014). Considering the resemblance of the climatic conditions and husbandry practices, these differences could be attributable to a more intensive therapeutic or prophylactic veterinary intervention, a higher level of herd immunity or host resistance, a lower density of infective stages, or a combination of these factors.

Strongyle eggs were present in 99.1% (**Table 6**) of all positive faecal samples. This high proportion of Strongyle is frequently reported in tropical areas (Mohma et al., 2001; Maichomo et al, 2004; Olanike et al., 2015). In Mozambique, data on the epidemiology of gastrointestinal parasites are scarce, but the following Strongylida parasites were reported from descriptive studies in goats: *Trichostrongylus axei, Trichostrongylus falculatus, Trichostrongylus colubriformis, Strongyloides papillosus, Protostrongylus rufescens,*

Oesophagostomum columbianum, Haemonchus contortus, Gaigeria pachyscelis, Skrjabinema ovis, Trichuris ovis, Trichuris globulosa, Cooperia curticei, Cooperia pectinata and *Cooperia punctata* (Cruz e Silva, 1971; Specht, 1982; Atanásio, 2000). In this study, parasites from the Strongylda order were not identified to species level, but according to Specht (1982) and Atanásio (2000), *H. contortus*, *O. columbiano* and *T. colubriformis* are the most prevalent species in Mozambique. The presence of *H. contortus* is commonly associated with acute haemorrhagic anaemia due to the hematophagous nature of this nematode. . Haemonchosis could also present a chronic course and the main clinical signs are: anaemia, submandinbular oedema, ascites, lethargy, dark faeces, progressive weight loss and weakness (Taylor et al., 2007). *Oesophagostomum sp.*, also designated as "nodular worm", is commonly associated with typical nodules in the small and large intestines. Severe dark green or intermittent diarrhoea, anaemia, rapid weight loss, emaciation and death in young animals are the main clinical symptoms of this parasitic infection (Stewart and Gasbarre, 1989). In the present survey, 87% of the goats presented *Oesophagostomum spp*. nodules. This proportion is just slightly higher than the 71.3%, reported by Nwosu et al., 2013, in a study conducted in a Nigerian abattoir on natural infected goats. These results emphatically indicate the importance of *Oesophagostomum spp*. in Tete Province.

Cestode parasites were also observed in our study (*Taenia hydatigena* cysts, eggs of *Moniezia spp*. and adult tapeworms of *Stilesia hepatica*) illustrating the goat parasitic diversity in Tete. These parasites had been previously recorded in Mozambique by Cruz e Silva (1991), Specht (1982) and Atanásio (2000). The prevalence of *Moniezia spp*. in tropical areas differs according to the region and the time of the year. The prevalence of the infections found in this study was 14.5%. Similar surveys conducted in Ethiopia, India, Kenya and Nigeria show prevalence rates ranging from 5% to 40% (Maichomo et al., 2004; Pathak and Pal, 2008; Tafera el al., 2009; Owhoeli et al., 2014). Differences in prevalence could be associated to different factors such as, treatment of definitive host, presence of *Oribatidae* mites, seasonal variation and host age. Higher *Moniezia spp*. prevalences have been consistently observed during the summer (Pathak and Pal., 2008). *Moniezia spp*. infection in goats is normally associated with unthriftiness, diarrhoea, respiratory signs and even convulsions (Elliot, 1984). However, Dever et al. (2015), in a study conducted in Northern South Wales, Australia, found that the removal of *Moniezia spp*. did not affect the growth rate in meat-breed lambs.

Taenia hydatigena infections in young animals are associated with poor body condition and emaciation due to the migration of immature forms through the hepatic parenchyma for approximately 4 weeks. In adults, the infection is usually asymptomatic and the abattoir condemnation of affected tissues is commonly reported. There are no clinical symptoms related to the infection with *S. hepatica*. However, the parasite in heavily infected animals may cause bile duct obstruction.

Coccidiosis is a high prevalent enteric disease caused by a protozoan parasite belonging to the Eimeria genus, which affects a variety of animal species, including small ruminants. Eimeria parasites are strictly host-specific. In goats, 13 species of Eimeria have been reported from different regions of the world (Chhabra and Pandey, 1991).The high prevalence (89%) of Eimeria infection in goats found in this study is consistent with observations made in other tropical countries, where the values of prevalence for this genus varies between 88.7% and 100% (Chhabra and Pandey, 1991, Kusiluka et al., 1996, Jalila et al., 1998, Harper and Penzhorn, 1999, Cavalcante et al., 2012). Coccidiosis is typically a disease of intensification, where the young animals are the most affected group and the main clinical signs are loss of appetite, unthriftiness, and profuse watery diarrhoea (Chartier and Paraud, 2012).

Parasitic disease continues to be a major constraint for livestock production. The occurrence of parasite co-infections, observed in 90% of our samples (Table 7), indicates a complex level of host-parasite interaction, where the host is exposed to different pathogenic factors generated by different parasite species. Usually, gastrointestinal parasites are not associated with high mortality. Their negative impact is commonly related to chronic wasting conditions, where production and productivity losses are chief objective indicators. In order to decrease the harmful effects on animal production, prevention measures should be implemented, taking into consideration the five check out procedures to control/prevent internal parasites in small ruminants in South Africa proposed by Bath (2014) namely; host resistance and resilience, reducing parasite load, evaluating pasture factors, evaluation the parasitic burden and optimizing drugs to be used. Provincial veterinary services, in collaboration with research institutes and universities, should define appropriated control measures for gastrointestinal parasites. During the implementation phase, it will be important to consider awareness campaigns towards improved animal husbandry and animal welfare, through the use of relevant educational campaigns involving smallholder goat producers.

5.2. Morphological characterization of *Taenia multiceps* larvae

In Africa, *T. multiceps* cysts in the central nervous system (CNS) have been reported in domestic ruminants in Kenya, Ethiopia, Democratic Republic of Congo, Senegal, Sudan, Chad, Angola, South Africa (Urquhart et al., 1996) and Mozambique (Vink et al., 1998). However, none of these studies contain information regarding the predilection sites of larval stages. The prevalence of *T. multiceps* cysts in naturally infected goats in this study (14.8%) was higher than the prevalence reported in a retrospective study in sheep in Ethiopia (2.34-4.54%) (Achenef et al., 1999) as well as that found in a prevalence study in goats in Gaza (8%) and Tete (7.3%) provinces of Mozambique (Atanásio, 2000). The prevalence obtained in this study was slightly higher than the prevalence reported by Vink et al. (1998) (13.8%) in a study conducted in Tete abattoir. However, higher prevalence rates were reported for coenurosis in Turkey (Uslu and Guclu 2007) and in slaughter slabs in Nogorongoro, Tanzania (Miran et al., 2015), 36.8 and 44.4 % respectively. Different factors could explain the variability in prevalence, as among them, the nature of the production system and the deworming of definitive hosts are considered the main ones.

The commonest predilection site of *T. multiceps* cysts in sheep is the CNS, whilst in goats, apart from the CNS, the presence of cysts have been reported in muscle, subcutaneous tissues, abdominal cavity, diaphragm, liver, kidney, heart, lymphatic nodules, tongue and eyes (Soulsby, 1982; Sharma and Chauhan, 2006; Islam et al., 2006; Godara et al., 2011; Schuster et al., 2010; Oge et al., 2012; Kheirandish et al., 2012; Monsang et al., 2014). Hago and Abo-Samra (1980) classified subcutaneous cysts in an infected goat as *Multiceps gaigeri* and in subsequent studies similar cysts were found in several organs including muscles and subcutaneous tissue and were reclassified as *T. multiceps* (Nooruddin et al., 1996; El Sinnary et al., 1999; Sharma and Chauhan 2006).

Results from our study show that a larger proportion of *T. multiceps* cysts were found outside the CNS and mostly in muscle and subcutaneous tissues in the experimentally infected goats than in the naturally infected ones. This difference is likely to be due to the fact that the cysts are commonly located in muscle medium and deep layers, which increases their possibility of being unnoticed at abattoir inspection stage. The low sensitivity of abattoir routine meat inspection for *T. multiceps* cysts contributes to the perpetuation of the parasite in the cycle (Sharma and Chauhan 2006)

Previous experimental infections with *T. multiceps* larva were conducted in sheep at a dose of 5500 and 6500eggs per sheep (Biyikoglu and Doganay, 1998; Ghazaei, 2007). In the two experiments the infection rate was 100 % in contrast to the lower infection rates (78.6 and 82.8% for experiment 1 and 2 respectively) recorded in our study. This difference might have been associated to the combination of one or more of the following factors: a relatively low infection dose, egg viability, or regurgitation of the infected bolus.

Previous studies show that clinical coenurosis in sheep is common in young animals (Abo-Shehada et al., 2002; Scala et al., 2007). Varcasia et al. (2009) suggested an age-related resistance to infection with *T. multiceps* in sheep, however, the mechanism by which this may come about is not apparent and cross immunity within Taenidae tapeworms could be a possible mechanism of resistance (Edwards and Herbert, 1982). The increase in cyst size with age and number of scoleces found in this study are in accordance with a previous study in sheep by Willis and Herbert (1987).

Our findings have shown that predilection sites of *T. multiceps* cysts in goats apart from the brain include skeletal muscles and subcutaneous tissue, lungs, diaphragm, heart and mesentery. Furthermore, they have also shown that the current routine abattoir inspection targeting only the brain is not a reliable method in detecting all cases *T. multiceps* infection in goats. Development and validation of a sensitive and specific diagnostic method for *T. multiceps* infection in goats should be investigated in order to improve the surveillance and control of the parasite.

5.3. Molecular characterization of *Taenia multiceps* from Mozambique

A thorough search of African scientific literature revealed that *Taenia multiceps* larva are present in small ruminants in Kenya, Ethiopia, Congo/Zaire, Senegal Sudan, Chad, Angola, South Africa, Namibia and Mozambique (Boharmann, 1990; Urquhart et al., 1996; Vink et al., 1998; Achenef el al., 1999), but information regarding to the goagraphical distribution in all country and strain characterization was not provided. In Mozambique this parasites was been report in goats (Vink et al., 1998; Atanasio, 2000). Our study aims at determining the molecular characterization of *T. multiceps,* which infect goats from Mozambique and represents one of the first investigations of this type in the country.

Firstly, all analysed sequences showed highest homology with *T. multiceps*. These results also supported high homology to the ND1 and COI mitochondrial genes within the cestodes

as previously described (Gasser et al. 1999; Varcasia et al., 2006: Collomb et al., 2007). Pairwise comparison COI sequences of *T. multiceps* showed lesser degree of variability than ND1 sequences when compared with others deposited in GenBank (Varcasia et.al. 2006; Oryon et al., 2010; Avcioglu et al., 2011). The ND1 gene sequences seem to be a more powerful target for the species identification and to investigate population variations within *T. multiceps* as previously suggested by Gasser et al., (1999). Moreover, COI sequences present more similarities between *T, multiceps* and *T. serialis* than the ND1 sequences.

When we compared the sequences studied with the GenBank not observed any clustering in thier location of the cyst. What suggests to us that there Differences Between the sequences of brain cysts and noncerebral

When compared, both ND1 and COI gene sequences from our study and other *T. multiceps* sequences deposited in the GeneBank did not observed any clustering related to cyst location. This suggests that no differences between gene sequences between cerebral and non-cerebral cysts. For ND1 gene sequences most of the cysts (7/15) are between GeneBank access code DQ309770 from a cerebral sheep cyst reported in Italy (Tm2) (Varcasia et al., 2006) to HM101470, a cyst from goat muscles in Iran (Oryom et al., 2010), 5/15 above HM101470 and finally 3/15 between Tm3 from Sardine to Wales *T.multiceps* sequence (Gasser et al., 1999). Consequently, for ND1 the studied samples do not showed any particular clustering position, which indicating no association between genetic variability and host species or geographical region. The COI gene sequences seem to discriminate among different geographic locations. For this marker, isolates from Asia and Middle East form a group, which is slightly different from European isolates. For both genes; ND1, COI does not observed any genetic variation associated to the host species.

The results of the present study are in agreement with previous studies in which *Taenia multiceps* is referred to as responsible for the formation of cerebral and non-cerebral cysts (Rostami et al., 2013; Akbari et al., 2015: Amrabi et al., 2015). Nevertheless, the number of sequences analysed are not enough to extrapolate the present results to Mozambique or Africa. Further work should be done comparing parasites from diverse geographical regions in Mozambique and in Africa as well as from different hosts.

5.4. Antigenic characterization of *Taenia multiceps* larvae

Taenia multiceps larva is an important parasite affecting ruminants and rarely in humans, is the disease, coenurosis, an important condition on animal health (Ghazei, 2007). For an effective control a precise diagnosis of coenurosis in goats is essential. Immunodiagnositic as been used as a tool for aid in auxiliary diagnostic, confirmation test, management treatment of diseases and for epidemiological studies (Dorny at al., 2003; Ortona et al., 2003; Zhang et al., 2007). To standardize a immunodiagnositic it is essential to know the antigenic characterization of the pathogen. The objective of this study was characterizing the antigens of *Taenia multiceps* larva.

From the produced antigen higher concentration of proteins were found in those prepared on the basis of fluid and membrane of the cyst (AgFE and Ts) probably due to the fact that protein are present in cyst fluid as well as in the membrane. Antigen with lower concentration protein was these produced oncosphere this condition could be related with insolubility in PBS following lysis by sonication of some proteins as recent reported by Li et al. (2014).

Analysing of polypeptides in SDS-PAGE gel in five fluid, fluid and membrane and oncosphere from *T. multiceps*, *T. solium* and *T. hydatigena*, antigens reveled a complex protein bands pattern with a appearance of almost similar to AgFe and Th (11 bands). While, in AgO only two bands were observed. Cannot draw any conclusions from this, fact given the the fact that little amount of proteins in AgO. Most of the bands were allocated between 90 Kda to 66.5 Kda similar results was obtained by Price et al. (1989) with *T. multiceps* crude cyst fluid.

In western blot, positive sera were detected on bands allocated below 26.5 KDa and between 36.6 – 48.5 KDa for 30 and 45 days after experimental infection respectively. In western blot using chemiluminescent a very bard band was observed 165 days after infectionwith AgF between 40 to 15 KDa.

When evaluating the presence of antibodies and antigens in 5 animals infected with different parasitic burden (0 to 16 cysts per animal) no relationship between presence of antibodies and number of parasites were observed. However, in the negative animal no positive antigen values were observed when using the monoclonal antibody HP10. This confirms the need to use more specific fractions in order to detect the presence of infections *Taenia multiceps* in ruminants.

AThe lack of accuracy in detecting early infections with *T. multiceps* demonstrated the lack of sensitivity and specificity of antigens used. The same problems found with other metacestodes; cross reaction within *Taenia* species, lack of sensitivity and specificity. Following the same diagnosis steps with other well-known metacestodes recombinant protein and syntectic pepetides could be developed to achieved more sensitive and specificity test.

5.5. Pharmacokinetic of albendazole in goats

Anthelmintic has been used at animal production such as prophylactic or as treatment. Factors which influence pharmacokinetics in domesticated animals must be taken into consideration in order to maximize the efficacy of these drugs. Age, genetics, clinical conditions and dosage are some of these factors which influence the pharmacokinetic profile; the objective of this study was to analyse the effect of these three factors on the plasma disposition of ABZ metabolites in indigenous goats from Mozambique.

Pharmacokinetic behaviour of ABZ has been studied in different species including rat (Fargetton et al., 1986; Delatour et al., 1991b), mouse (Douch and Buchanan, 1979), rabbit (Li te al., 1995), poultry (Li et al., 1995), sheep (Marriner and Bogan, 1980), goat (Delatour et al., 1991a, Sanyal, 199), cattle (Prichard et al., 1985), pig (Souhaili-El Amiri et al., 1987), dog (Delatour et al., 1991b) and man (Penicaut et al., 1983). These authors reported similar pharmacokinetic profiles to those observed in the present study. Parent drug (ABZ) was absent or in very small amounts in plasma. ABZ is rapidly absorbed in the gastrointestinal tract and it is known to suffer a first pass effect, and it is rapidly metabolized in the liver transforming into ABZSO and $ABZSO_2$. Gastrointestinal and hepatic metabolisms are involved in the biotransformation of ABZ. Gastrointestinal fluids and enterocytes are responsible for the BZD metabolism in the gastrointestinal tract (Lanusse and Prichard, 1993). Oxidation of ABZ to ABZSO at intestinal level occurs with the participation of flavin-containg monoosygenase (FMO) (Galtier et al., 1986, Lanusse et al., 1993, Moroni et al., 1995) and cytochrome P-450 (CYP) (Souhaili-El Amiri et al., 1987) enzymatic systems. Previous studies into liver and intestine microsomes of rat have demonstrated that both systems are similarly involved (Moroni et al., 1995, Redondo et al., 1999). It was observed *in vitro*, in intestinal microsomal from rats, that ABZ were captured by enterocytes and then metabolized into ABZSO (Villaverde et al., 1995). However, the main biotransformation of ABZ is performed in the liver, where successive oxidation leads to ABZSO and $ABZSO_2$ (Lacey, 1990; Lanusse and Prichard, 1993). Liver microsomal fraction: the flavine

monoxygenase (FMO) (Fargetton et al.; 1986, Morini et al., 1995) and cytochrome P-450 (CYP) (Souhaili-El Amri et al., 1988b) enzymatic systems are responsible for the biotransformation of ABZ. FMO and CYP3A participate in the first metabolic step, from ABZ to ABZSO, whereas the formation of the sulfone is mainly catalysed by the CYP1A subfamily (Souhaili-El Amri et al., 1988a). These findings are supported by the fact that after administration of ABZ in sheep and cattle the peak plasma concentration of $ABZSO_2$ metabolite was delayed compared with that of ABZSO (Marriner and Bogan, 1980, Hennessy et al., 1989). Identical results were observed in our study, where plasma concentrations of ABZSO were higher than $ABZSO_2$, in the period of 12 to 24 hours post-administration, and the plasma concentrations of $ABZSO_2$ increased reaching the maximum concentrations after 20-24 hours post-administration.

When comparing the kinetic profile of adult male healthy goats with young animals after an administration of a single dose of ABZ, it was observed that ABZSO plasma concentrations profile was higher in adult than in young animals (**Figure 16**). The differences in the pharmacokinetic behaviour between groups may be influenced by the enzymatic metabolism pathway, since the efficacy of some oxidative pathways increased with the age. It is known that some metabolic pathways are developed with age, and in particular it is observed with hepatic oxidative pathways. In general, individual newborns have deficit enzymes involved in non-specific hepatic metabolism. This may be the case of the oxidative pathways responsible for ABZ metabolism. The metabolic processes mature with age and therefore metabolism is slower in young animals than in adults.

For ABSO2 no differences were observed. This could be explained by the fact that the metabolism from ABZSO to $ABZSO_2$ could be saturated and therefore the differences were not observable.

On the other hand, the rumen plays a special role in the disposition of drugs absorbed from the gastrointestinal tract. In adult animals, ABZ is diluted throughout the rumen, which acts as a large reservoir of the drug, delivering it slowly. However, at normal rumen pH (6.5-7.0), the solubility of benzimidazole compounds is low, being decreased substantially at the lower abomasum and upper small intestine (pH =2-3), favouring absorption (Steel and Hennessy, 1999). In young ruminants, the oesophageal groove acts as a bypass of the drug directly into the abomasum, increasing the gastrointestinal transit and decreasing the disposition of low

soluble drugs, such as ABZ. These facts may also explain the differences between ages observed in this study for both metabolites (**Table 12**).

In parallel, it has been observed that ABZSO and $ABZSO_2$ Tmax and MRT were significantly shorter in male and female young animals. The results described herein clarify the importance of the rumen in the kinetics of ABZ metabolites and explain the age differences observed in the present study.

McKellar et al. (1993, 1995) did not observe significant differences between 1 and 8 month old and between 3 and 9 month old animals when ABZ, ABZSO and $ABZSO_2$ were administered. Meanwhile, differences in some pharmacokinetic parameters were observed when NTB was administrated to 3 and 9 month olds (McKellar et al., 1993). However, in this study, they assessed the pharmacokinetic profile in animals with very close ages, 3 and 8 months old, while in the present study the animals were more distant, between 4 months and 2-3 years old. This aspect could also explain the observed differences between the two studies.

These results suggest that the adult dose schedule should be modified in young animals in order to increase the bioavailability and enhance the time in which the drug-parasite interaction takes place. These results suggest the need for further study of the role of the gastrointestinal metabolism and digestive anatomical differences between adult and young animals for the Benzimidazole drugs.

No differences were observed in the case of the kinetic profile of ABZSO and $ABZSO_2$ obtained in female and male after treatment of ABZ, while only significant differences between sexes were observed for Tmax. Cristofol et al (1998) in sheep treated with Netobimin (ABZSO), observed differences in ABZSO and $ABZSO_2$ kinetic parameters between male and female, and males were the ones who eliminated more rapidly.

Pharmacokinetics in *Taenia multiceps* infected animals

It is known, that in some cases, differences were observed in the kinetic parameters of the drugs, when these were administered to animals of the same species, age and sex, who were healthy or sick.

In the present study, the pharmacokinetic profile of ABZ and metabolites were evaluated in goats experimentally infected with *T. multiceps* after treatment with two different doses of

ABZ. Plasma concentrations of ABZSO, $ABZSO_2$ and ABZ followed a similar pattern of distribution to that previously described in healthy animals.

ABZ is present in plasma at low concentrations in both administered doses (10 and 20 mg/kg). In healthy animals, ABZ was not detected in plasma. This could suggest a modification of gastrointestinal availability and intestinal and hepatic metabolism of ABZ. These changes allow greater permanence of ABZ in the gut and in the liver and could cause a delay in the biotransformation of ABZ into ABZSO.

Significantly higher Cmax and AUC values for ABZSO and $ABZSO_2$ were observed after oral administration of 10 mg/kg of ABZ to healthy adult goats compared with the same dose to *T. multiceps* infected animals. When data from $ABZSO_2$ kinetics were analyzed in infected animals, it was possible to detect a delay in the synthesis of this metabolite and extension of its presence. These data suggest that the disease causes a decrease in the formation and elimination of ABZ-metabolites.

It has been reported that diseases or infections could change drug pharmacokinetics and biotransformation. Liver damage caused by parasitosis usually decreases the ability of the liver to metabolize drugs due to a reduction of enzyme activity and hepatic blood flow. It has been demonstrated, in infected sheep with *F. hepatica*, that sulphonization of ABZSO in hepatocites was reduced and there was also a significant decrease of activity level for CP450, but the FMO activity remained unchanged (Galtier, 1991). A decrease of CYP3A expression and the activity of hepatic microsomes was observed in studies with *F. hepatica* infected sheep (Jemili et al., 1994; Calléja et al., 200). However, a significant increase in enzymes related to FMO and a significant decrease of glutathione-*S*-transferase (GST) activity was observed in mouflon (*Ovis musimon*) with *Dicrocelium dendriticum* infection after administration of ABZ (Skálová et al., 2007). An inductive effect on P4501A was observed in mouflon (*O. musimon*) parasited with *Dicrocelium dendriticum*, after administration of a single dose of ABZ (Lamka et al., 2007). In rats, infected with *F. hepatica*, a decrease of activities of drug metabolizing enzymes and an increase of activities of CYP3A and CYP1A were observed (Biro-Sauveur et al., 1995). Liver damage caused by oncosphere of *Taenia multiceps*, in goats, occurs during larva the migration before cyst development. There are no reports on liver damage associated with *T. multiceps* infection in small ruminants. In a study with naturally infected goats Christodoulopoulos et al. (2015) found significantly higher values of aspartate amino transferase, within normal range, when compared with uninfected

goats. However, these findings were attributed to the fact that animals had parasites. The fact that *T. multiceps* animals presented low ABZ metabolites may be associated to the presence of gastrointestinal parasites, considering that infected *T. multiceps* animals were treated with anthelmintics two months before infection. Previous studies demonstrated that the presence of gastrointestinal parasites could reduce the absorption and distribution of BZD compounds due to changes in abomasal pH, mucosal permeability and intestinal atrophy (Sykes, 1978; Dakkak, 1984; Alvarez et al., 1997).

With the aim of analyzing the availability in infected goats of ABZ and their pharmacologically active metabolites, the kinetic profiles of two different doses of ABZ were studied. Later, with the obtained results, the best single dose was selected and another study was performed to compare the single with multiple dose treatment. With the pharmacokinetic results of these treatments, the most appropriate ABZ treatment for the infected goats was decided.

After oral administration of 20 mg/kg of ABZ to infected male goats, the ABZSO is detected in plasma quickly, reaching maximum plasma concentrations around 18-24 h. Plasma concentrations were higher and remained in plasma for a longer time than the data observed after oral administration of a dose of 10 mg/kg. These results agree with the fact that an increase in dose should result in an increase of plasma concentrations and AUC. For the $ABZSO_2$, Cmax, AUC, Tmax and MRT were higher in goats treated with 20 mg/kg of ABZ than goats treated with 10 mg/kg. Conversely, no significant differences are found in T1/2 of the elimination terminal phase. These data suggest that the dose of 20mg/kg can achieve higher and sustained levels of pharmacologically active metabolites and thus can ensure greater efficacy and efficiency of treatment.

However, when doses of 10 or 20 mg/kg of ABZ were administered to infected goats, no significant differences were observed in efficacy between both doses. These data are discussed in the next section of clinical efficacy of ABZ.

As the efficacy results of the single oral dose of 20 mg/ kg of ABZ did not show greater effectiveness than the dose of 10 mg/kg, a higher multiple dose dosage was given. An oral dose of 10 mg/kg of ABZ, every 24 h, for three consecutive days was applied. The plasma concentrations of ABZSO and $ABZSO_2$ show that the infected goats remain under the effect of these molecules for over 90 hours and high concentrations over 72 hours.

The kinetic profile of multiple doses was analyzed and it was observed that after the first dose the concentration of ABZSO was higher than that of $ABZSO_2$ but in the second and third administrations, $ABZSO_2$ plasma concentrations increased and were higher than those observed for ABZSO. For ABZSO, the high plasma concentrations were observed around 9-12 h, 30-36 h and 54-63 h after the first, second or third administration, respectively. Meanwhile, maximum plasma concentration for $ABZSO_2$ was observed slightly later, at 15-21 h, 33-45 h and 60-66 h after the first, second or third administration, respectively. After 60 h for ABZSO and 63 h for $ABZSO_2$, respectively, plasma concentrations decreased, and a rapid elimination phase was observed for both.

The comparison of the respective plasma concentrations obtained after each of the 3 doses, shows that the ABZSO reached the steady state after the first dose (Cmax and AUC were similar for the 3 doses). Conversely, plasma concentrations of $ABZSO_2$ growing from first to third doses, reaching a higher Cmax and a higher AUC with each dose. This would agree with the inducer effect of ABZ on the hepatic oxidative metabolism, described by Delatour et al. (1990) in sheep and Souhaili-EI-Amri et al. (1988) in rats, which would increase the metabolism of ABZ to ABZSO, and ABZSO to $ABZSO_2$.

Moreover, an increase in the liberation of ABZSO may be produced via the biliary gland to the intestines and this metabolite would then be reduced to ABZ due to the presence of gastrointestinal fluids. (Lanusse et al.,1992 a,b and Capece et al, 2001). This suggests that higher concentrations of ABZ could be found in the intestine which would facilitate the elimination of these intestinal parasites. Therefore, lower concentrations were observed for ABZSO than for $ABZSO_2$.

These data suggest that this dosing regimen can cover, from the therapeutic point of view, better than single doses the infected animals. Plasma concentrations observed for both metabolites, taking into account the molecular evaluation, are similar to those obtained with a single dose of 20 mg/kg, but the parasite remained in contact with the drugs a longer period of time.

Another important point in the analysis of the efficacy of ABZ against *T. multiceps larvae*, is to know whether or not the drug and its metabolites reach the places where the parasite is found in the tissue of the infected goats. To this end, the presence of ABZ and its metabolites

were studied in plasma, brain, muscles and cyst fluid in infected male goats, 12 h after an oral administration of 10 mg/kg of ABZ.

Twelve hours after the treatment, the plasma concentrations were 2.0±0.9 μg/ml and 0.6±0.2 μg/ml for ABZSO and $ABZSO_2$ respectively. At the same time, the goats were sacrificed and brain, muscles and fluid cyst were collected. ABZ appeared in the highest concentrations in muscles followed by cyst fluid, brain and plasma, 0.77±0.53 μg/g, 0.40±0.01 μg/ml, 0.19±0.09 μg/g and 0.02±0.03 μg/ml, respectively.These data suggest that the ABZ and its metabolites reach the tissues where the parasite is located and that the ABZ and ABZSO molecules, through great anthelmintic activity, would be in condition to eliminate the parasite.

5.6. Efficacy of albendazole against *Taenia multiceps* larva in goats

Surgical removal and anthelmintic administration were reported in the literature as the treatment of *Taenia multiceps* cysts. However, surgical removal, the most used method in practice, requires accurate localization and the success rate can reach up to 74% (Skerrit and Stallbaumer, 1984; Tirgari et al., 1987; Komneou et al., 2000). Praziquantel, albendazole, febendazole and drug combinations have been administered on an experimental scale in acute coenurosis in sheep with efficacies ranging from 54.7% to 100% (Verster and Tustin, 1982; Biyikoglu and Doganay, 1998; Ghazaei, 2007).

The effectiveness against the larvae of *Taenia multceps* data observed after treatment of infected goats (2 months post-infection) with ABZ at oral single dose of 10 o 20 mg/kg, or at multiple doses of 10 mg/kg in 3 consecutive doses, shows that the treatment with multiple doses was the most effective for the viability of cysts. The single doses 10 and 20 mg/kg presented lower efficacy and no differences were observed between both doses.

Therefore, it can be concluded that the therapeutic effect of ABZ on the elimination of *T. multiceps* larvae is time dependent and that once they are reached in the place of infection it is necessary that the therapeutically effective concentrations of ABZ and its metabolites remain in place for enough time to eliminate the parasite.

These results are in accordance with the data obtained by Delatour et al., (1990) and Lanusse and Prichard, (1993). Another possibility that is linked with the efficacy of multiple doses of ABZ is the action mechanism of BDZ compounds which include the enzymes inhibition

related to energy metabolism (Behm and Bryant, 1979). In fact, when the time of exposure to an anthelmintic increases, the ability of the parasite to use reserved energy will be compromised (Prichard el al., 1978). Previous studies carried out on goats, cattle and buffalo have demonstrated that prolonged administration of ABZ increased the drug activity (Chartier et al., 1996, Sanyal, 1998) and allowed the extension of the drug/parasite interactions time and consequently their efficacy (Lanusse and Prichard, 1993).

According to the criteria of the World Association of Advanced Veterinary Parasitology, effectiveness of ABZ was observed in the multiple administration two months after infection. This approach of using multiple doses of ABZ has also been applied to humans against metacestodes of *Echinococcus granulosus* (Kern, 2006; WHO, 1996) and neurocysticercosis (Garcia et. al., 2003; Matthaiou et. al., 2008; Khurana et al. 2012) and yielded better results compared to a single dosage. Although the efficacy of 73.9% and 72.72% observed for single doses of 10 mg/kg and 20 mg/kg respectively in this study appear low compared to results observed in acute experimental coenurosis in sheep (Biyikoglu and Doganay, 1998), these differences could be explained by variation of cyst location. In our study, most of the cysts were located outside the brain in contrast with the observations by Biyikoglu and Dognay (1998) where all cysts were located on the brain. No conclusions on the efficacy of ABZ on cerebral cysts could be made from our study due to the fact that a small number of cysts were found in the brain (9 out of 153). Despite the fact that this study analysed the efficacy of three regimes, we considered the dose for cyst to be the most important due to the fact that *T. multiceps* larvae could infect humans. In relation to cysts in the brain, there are some disagreements on the effectiveness, Ghazei (2007) found that after treatment with ABZ at a dose of 25 mg/kg during 6 days, all cysts in the brain of sheep, (acute coenurosis) died or degenerated.

From the few cysts found in brain, in our study, it was observed that in the same animal one cyst was viable and the other was not. This varied nature observed in our study has also been observed in human with hydatid cyst treated with albendazole (Todorov et al., 1992) and no explanation was advanced to justify this finding.

Alvarez et al. (2012) observed that when increasing the ABZ doses there are also increases of bioavailabilty of ABZ metabolites and mean residence time in lambs and a positive correlation on efficacy against *Haemonchus contortus*. By contrast, in our study no significant differences on efficacy were observed in the two single doses of ABZ. In the study

of Alvarez et al. (2012) they used higher doses, between 5 and 45 mg / kg of ABZ, therefore higher dosages will give higher concentrations and residence times of the drug and metabolites in the body.

The results presented in this study have demonstrated that ABZ at a dose of 10 mg/kg for 3 days is effective in treating early infection of *T. multiceps* larva in goats. Nevertheless, with five months old cysts the number of non viable cysts in the treated group was similar to the control group with values of 88.6% and 84.6%, respectively (Table 20). These findings suggest that the lifespan of the parasite in muscular and subcutaneous tissues could be shorter than five months. There are no available studies on the lifespan of cysts of *T. multiceps* in goat muscles or subcutaneous tissues. Therefore, further work is required to understand the biology of *T. multiceps* larva in muscular and subcutaneous tissues.

The present work on the efficacy of ABZ in 3 therapeutic regimes on two and five months olds *T. multiceps* cysts in goats makes a contribution towards the knowledge of the best strategies for the treatment of asymptomatic coenurosis.

CONCLUSIONS

6. Conclusions

1. A High prevalence of different parasites in goats slaughtered at TeteMunicipal abattoir was observed, strongly indicating that Strongylyda, *Taenia hydatigena* larva, *Taenia multiceps* larva, *Moniezia spp*. and *Eimeria spp*. play an important role in goat health and production.

2. The overall prevalence of *Taenia multiceps* larvae in slaughtered goats at Tete Municipal abattoir was 14.8%.

3. Goat infection rates after experimental infection with *Taenia multiceps* were 78.6% and 82.8% respectively, for experiments performed in two different times.

4. On natural and experimental infections, subcutaneous and muscular tissues were the most common locations for *T. multiceps* larva. It was also found that the brain, mesentery, diaphragm, lungs and heart were infected by *T. multiceps* larva.

5. After COI (cytochrome c subunit 1) and ND1 (NADH dehydrogenase 1) mithochondrial gene sequence analysis in 15 cysts from naturally infected goats, no genetic differences were found between *Taenia multiceps* cysts with cerebral and non-cerebral localization. This fact confirms that the parasite found at both sites was the same.

6. The results from the antigenic characterization study corroborate the suggestions from previous studies reporting the difficulty to develop immunoassys of practical use based on crude or SDS-PAGE fractioned antigens. More purified antigens must be used in order to develop an immunoassay that would be able to detect *Taenia multiceps* infections at early stages.

7. The Kinetic profile of ABZ and the metabolites ABZSO and $ABZSO_2$ in healthy and infected animals were similar and agree with other studies described in literature.

8. In healthy goats, age differences in the plasma profiles of ABZ metabolites were observed. Adult males presented higher plasma concentrations than young animals after oral administration of 10 mg/kg of ABZ. No sex-related differences were observed for the studied ABZ metabolites.

9. The pharmacokinetic parameters of ABZSO and $ABZSO_2$, from infected and healthy goats treated with 10 or 20 mg/kg of ABZ, showed statistically significant differences in Cmax, Tmax, AUC and MRT.

10. Lower Cmax and AUC for both metabolites were observed in *T. multiceps* infected goats when compared with healthy goats, after oral administration of 10 mg/kg of ABZ. This suggests that the infection with the larval stage of *T. multiceps* causes pathophysiological changes that alter the kinetics of ABZ and metabolites.

11. In a multiple ABZ dose (3x10mg/kg), ABZSO appeared earlier and in higher concentrations than $ABZSO_2$ in the first 24 h, but after 48 hours, the plasma concentrations of $ABZSO_2$ were greater than ABZSO.

12. When comparing the first 24 hours in *T. multiceps* infected goats after a single oral dose of 10 mg/kg ABZ with oral multiple doses of ABZ (3 x 10 mg/kg), Cmax and AUC were higher in the multiple dose treatment . However, no significant statistical differences between two groups of ABZSO kinetics parameters were observed. The high variability among individuals might be at the origin of the non-significant differences.

13. It has been observed that ABZ and its metabolites reached *T. multiceps* cysts. Twelve hours after the administration of an oral dose (10 mg/kg), ABZ, ABZSO and $ABZSO_2$ concentrations were highest in muscles, followed by the cyst fluid, the brain being the tissue were the lowest concentration was found.

14. When compared to the controls (animals without treatment), Albendazole caused a significant difference in cyst viability when it was used to treat *T. multiceps* cysts in goats 2 months after an experimental infection. No significant difference in cyst viability was observed between infected animals treated with multiple (3x10 mg/kg) or single (10 mg/kg) doses, or when the goats were treated with a single dose of 10 or 20 mg/kg of ABZ.

15. The percentages of non-viable cysts for the groups treated with a multiple doses of ABZ or treated with single doses (10 or 20 mg/kg) were 90% and 73%, respectively. The difference between these two percentages are not statistically significant. However, it is possible that the small number of animals used in the study was not enough to highlight this issue.

16. In animals infected with *T. multiceps* cysts treated 5 months after infection with a multiple dose of ABZ at 10mg/kg for 3 consecutive days, no differences were observed in cyst viability between the treated and non-treated control groups.

REFERENCES

7. References

Abo-Shehada, M. N., Jebreen, E., Arab, B., Mukbel, R., Torgerson, P. R. (2002). Prevalence of Taenia multiceps in sheep in northern Jordan. *Preventive Veterinary Medicine*. 55:201-207.

Achenef, M., Markos, T., Feseha, G., Hibret, A., Tembely, S. (1999). Coenurus cerebralis infection in Ethiopian highland sheep: incidence and observations on pathogenesis and clinical signs. *Tropical Animal Health and Production*. 31:15-24.

Adane P., Kumsa B., Hiko A., Afera B. (2015). Prevalence of Coenurus cerebralis in Small Ruminants Slaughtered at Hashim Export Abattoir, Debre Zeit, Central Oromia. European Journal of Applied Sciences. 7: 56-63.

Akal E., Akyurek, O., Can, E.S., Gunes, F., Altinbas, M. (2008). Albendazole-induced bicytopenia: case report. Turkish Journal of Hematology. 25:45-47.

Akbari M., Moazenia M.,Oryan A., Sharifiyazdic H.,Amrabadi O. 2015. Experimental cerebral and non-cerebral coenurosis in goats: A comparative study on the morphological and molecular characteristics of the parasite. *Veterinary Parasitology* 211: 201–207

Ali, D., Chick, B. (1992) Effect of feed type on the pharmacokinetic disposition of oxfendazole in sheep. *Research in Veterinary Science*. 52:382-382.

Alvarez, L., Suarez, G., Ceballos, L., Moreno, L., & Lanusse, C. (2012). Dose-dependent systemic exposure of albendazole metabolites in lambs. Journal of Veterinary Pharmacology and Therapeutics, 35 :365-372.

Alvarez, L. I., Sanchez, S. F., Lanusse, C. E. (1997). Modified plasma and abomasal disposition of albendazole in nematode-infected sheep. Veterinary Parasitology 69:241-53.

Alvarez, L. I., Saumell, C. A., Sánchez, S. F., Lanusse, C. E. (1996). Plasma disposition kinetics of albendazole metabolite in pigs fed different diets. *Research in Veterinary Science*. 60:151-156.

Alvarez, L., Sánchez, S., Lanusse, C. (1999). In vivo and ex vivo uptake of albendazole and its sulphoxide metabolite by cestode parasites: relationship with their kinetic behaviour in sheep. *Journal of Veterinary Pharmacology and Therapeutics*. 22:77-86.

Amrabadi, O., Oryan, A., Moazeni, M., Sharifiyazdi, H., & Akbari, M. (2015). Comparison of cerebral and non-cerebral coenurosis by genetic markers of glycolytic enzyme (enolase) and mitochondrial sequences in sheep and goats. Veterinary Parasitology. http://dx.doi.org/10.1016/j.vetpar.2015.10.021

Aminzhanov, M., Musinov, M., Baratov, V. A. (1988) Chemoprophylaxis of Coenurus cerebralis infestation in Sheep Using Febendazole and Praziquanel. Veterinarya. 10:46-47.

Anantaphruti,M., Thaenkham,U., Kusolsuk,T., Maipanich,W., Saguankiat,S., Pubampen,S. and Phuphisut,O. (2013).Genetic Variation and Population Genetics of *Taenia saginata* in North and Northeast Thailand in relation to *Taenia asiatica*. *Journal of Parasitology Research*. 310605

Asteinza, J., Camacho-Carranza, R., Reyes-Reyes, R. E., Dorado-Gonzáles, V., Espinosa-Aguirre, J. J. (2000). Induction of cytochrome P450 enzymes by albendazole treatment in the rat. *Environmental Toxicology and Pharmacology*. 9:31-37.

Atanásio, A. (2000). Helmints, Protozoa, Heartwater, and the effect of gastrointestinal nematodes on productivity of goats of family sector in Mozambique. PhD thesis, Medical University of Southern Africa, Medunsa, South Africa.

Avcioglu, H., Yildirim, A., Duzlu, O., Inci, A., Terim, K. K., & Balkaya, I. (2011). Prevalence and molecular characterization of bovine coenurosis from Eastern Anatolian region of Turkey. *Veterinary Parasitology*. 176: 59-64.

Baliharová, V., Velík, J., Fimanová, K., Lamka, J., Szotáková, B., Skálová, L. (2005). Inhibitory effect of albendazoele and its metabolites on cytochromes P450 activities in rat and mouflon in vitro. Pharmacological Reports. 75:97-106.

Bandyopadhyay, S., Devi, P., Bera, A., Bandyopadhyay, S., & Bhattacharya, D. (2010). Prevalence of gastrointestinal parasite in goats in Shillong, Meghalaya, India. WebmedCentral PARASITOLOGY 2010;1(9):WMC00777 doi: 10.9754/journal.wmc.2010.00777

Barragry T. 1994. Veterinary drug therapy. Lea and Febiger. London

Bath, G. F., & Van Wyk, J. A. (2009). The Five Point Check© for targeted selective treatment of internal parasites in small ruminants. *Small Ruminant Research*. 86: 6-13.

Behm, C. A., Bryant, C. (1979). Anthelmintic action - a metabolic approach (A review). Veterinary Parasitology. 5:39-49.

Benchaoui, H. A., Scott, E. W., McKellar, Q. A. (1993). Pharmacokinetics of albendazole, albendazole sulfoxide and netobimin in goats. *Journal of Veterinary Pharmacology and Therapeutics*. 16:237-240.

Benifla M., Barrelly R., Shelef I., El-On J., Cohen A., Cagnano E. (2007). Huge hemispheric intraparenchymal cyst caused by *Taenia multiceps* in a child. *Journal of Neurosurgery: Pediatrics*. 107: 511-514.

Benoit, E. (1992). Effect of repeated doses of albendazole on enantiomers of its sulfoxide metabolite in goats. American Journal of Veterinary Research. 53:1663-1665.

Berrozpe D., Lanao J.M.,. Plá-Delfina, J.M. (1997). Biofarmacia y Farmacocinética. Volumen I: Farmacocinética. Editorial Síntesis. Madrid.

Biro-Sauveur, B., Eeckhoutte, C., Baeza, E., Boulard, C., Galtier, P. (1995). Comparison of hepatic and extrahepatic drug-metabolizing enzyme-activities in rats given single or multiple challenge infections with Fasciola-hepatica. *International Journal of Parasitology*. 25:1193-1200.

Biyikoglu, G., Bagci, O., Oncel, T. (2001). A coenuriasis outbreak of sheep in Istanbul, Turkey. Pendik Veteriner-Mikrobioloji-Dergisi. 32:27-30.

Biyikoglu, G., Doganay, A. (1998). Effects of Praziquantel and Albendazole on Coenurus cerebralis in Experimentally Infected Lambs. *Turkish Journal of Veterinary and Animal Science.* 22:43-48.

Bogan, J. A., Marriner, S. E. (1985). The rumen as a pharmacokinetic compartment. *Veterinary Research Communication Proceedings, International Workshop*. Antwerp: 251-265.

Bohrmann, R. (1990). Coenurus in the Muscles of a Gemsbok (Oryx gazelle). *Veterinary Parasitology*. 36:353-356.

Bowles J, McManus D.P. (1993). Molecular variation in *Echinococcus. ActaTropica*. 53:291-305

Bowles J., Blair D., McManus D.P. (1992). Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. *Molecular and Biochemistry Parasitology*. 54: 165-173.

Brander, T. B., Pugh, D. M., Bywater, R. J. (1977). Veterinary Drug Therapy. Lea & Febiger.

Brooks D. R., Hoberg E.P. and Weekes P.J. (1991). Preliminary Phylogenetic Systematic Analysis Of The Major Lineages Of The Eucestoda (Platyhelminthes, Cercomeria). Proceedingsof the Biological Society of Washington, 104:651-668

Bussell, K. M., Kinder, A. E., Scott, P. R. (1997). Posterior paralysis in lamb caused by Coenurus cerebralis cyst in the lumbar spinal cord. Veterinary Record. 140: 560.

Bustos, A. J., Pretell, E. J., Llanos-Zavalaga, F., Gilman, R. H., Del Brutto, O. H., Gracia, H. H. (2006) Efficay of a 3-day course of albendazole treatment in patients with a single neurocysticercosis cyst. *Clinical Neurology and Neurosurgery*. 108:193-194.

Caglar, R., Yuzbaioglu, M. F., Bulbuloglu, E. (2008). In vitro effectiveness of different chemical agents on scolices of hydati cyst. *Journal of Investigative Surgery*. 21:71-75.

Calléja, C., Biogot, K., Eeckhoutte, C., Sibille, P., Boulard, C., Galtier, P. (2000). Comparison of hepatic and renal drug-metabolising enzyme activities in sheep given single or two-fold challennge infections with Fasciola hepatica. *International Journal of Parasitology*. 30:953-958.

Campbell, W. C. (1990). Benzimidazoles: veterinary uses. *Parasitology Today*. 6:130-133.

Capece, B. P. S., Casamiglia, S., Castells, G., Arboix, M., Cristòfol, C. (2001). Effects of ruminal microflora on the biotransformation of netobimin, albendazole, albendazole sulfoxide, and albendazole sulfoxide enentiomers in an artificial rumen. Journal of Animal Science. 79:1288-1294.

Capece, B. P. S., Navarro, M., Arcalis, T., Castells, G., Toribio, L., Perez, F.,Cristòfol, C. (2003). Albendazole sulphoxide enantiomers in pregnant rats embryo concentrations and developmental toxicity. The Veterinary Journal.165: 266-275.

Capece, B. P. S., Perez, R., Andaluz, A., Perez, F., Garcia, F., Castells, G., Cristòfol, C. (2002). Placental transfer of albendazole sulphoxide enantiomers in sheep. The Veterinary Journal, 163: 155-160.

Capece, B. P., Virkel, G. L., & Lanusse, C. E. (2009). Enantiomeric behaviour of albendazole and fenbendazole sulfoxides in domestic animals: pharmacological implications. The Veterinary Journal, 181: 241-250.

Capece, B. P. S., Castells, G., Pérez, F., Arboix, M., Cristòfol, C. (2000). Pharmacokinetic behaviour of albendazole sulphoxide enentiomers in male and female sheep. Veterinary Research Communication. 24:339-348.

Carpio, A., Escobar, A., Hauser, W. A. (1998). Cysticercosis and epilepsy: a critical review. *Epilepsia*. 39:1025-1040.

Casado, N., Rodrigues Caabeiro, F., Hernámdez, S. (1986). In vitro survival of Echinococcus granulosus protoscoleces. International Journal for Parasitology. 19, 945-947.

Cavalcante, A.C.R, Taeixeira, M., Monteiro, J.P., Lopes, C.W.G. (2012). Eimeria species in dairy goats in Brazil. *Veterinary Parasitology*. 183:356-358.

Chai, J., Menghebat, Wei, J., Deyu, S., Bin, L., Jincao, S., Chen, F., Xiong, L., Yiding, W., Guliber, Yanchun, W., Fanghua, G. Shuhua, X. (2004). Observations on clinical efficacy of albendazole emulsion in 264 cases of hepatic cystic echinococcosis. *Parasitology International*. 53:3-10.

Chartier C, Paraud C. (2012). Coccidiosis due to *Eimeria* in sheep and goats: a review. *Small Ruminant Research.*103:84 -92.

Chartier, C., Pors, I., Bernard, N.,Hubert, J. (1996). Efficacy of an albendazole slow-release capsule for the control of susceptible or resistant nematode parasites of dairy goats. Veterinary Parasitology. 67:197-206.

Chhabra, R.C., Pandey, V.S. (1991). Coccidia of goats in Zimbabwe. *Veterinary Parasitology*. 39: 199-205.

Christodoulopoulos, G. (2007). Two rare clinical manifestations of coenurosis in sheep. Veterinary Parasitology. 143:368 -370.

Chung, W.C., Fan, P.C., Lint, C.Y., Wut, C.C. (1991). Poor efficacy of albendazolle for the treatment of human taeniasis. International Journal for Parasitology 21:269-270.

Collomb, J., Machouart, M., Biava, M.F., Brizion, M., Montagne, K., Plénat, F. , Fortier, J. (2007). Contribution of NADH Dehydrogenase Subunit I and Cytochrome C Oxidase Subunit I Sequences Toward Identifying a Case of Human Coenuriasis in France. *Journal of Parasitology*. 93:934-937.

Cristòfol, C.; Carretero, A.; Fernandez, M.; Navarro, M.; Sautet, J.; Ruberte, J. & Arboix, M. (1995). Transplacental transport of netobimin metabolites in ewes. European Journal of Drug Metabolism and Pharmacokinetics. 20:167 171.

Cristòfol, C., Franquelo, C., Navarro, M., Carretero, A., Ruberte, J., Arboix, M. (1997a). Comparative pharmacokinetics of netobimin metabolites in pregnant ewes. Research in Veterinary Science. 62:117-20.

Cristòfol, C., Navarro, M., Franquelo, C., Valladares, J. E., Arboix, M. (1998). Sex difference in disposition of albendazole metabolites in sheep. *Veterinary Parasitology*. 78:223-231.

Cristòfol, C., Navarro, M., Franquelo, C., Valladares, J. E., Carretero, A., Ruberte, J., Arboix, M. (1997b). Disposition of netobimin, albendazole, and its metabolites in the pregnant rat: development al toxicity. *Toxicology and Applied Pharmacology*. 144:56-61.

Cristòfol, C., Virkel, G., Alvarez, L., Sánchez, S., Arboix, M., Lanusse, C. (2001). Albendazole sulphoxide enantiomeric ratios in plasma and target tissues after intravenous administration of ricobendazole to cattle. *Journal of Veterinary Pharmacology and Therapeutics*. 24:117-124.

Critchley, J., Addiss, D., Ejere, H., Gambleu, C., Garner, P., Gelband, H. (2005). Albendazole for the control and elimination of lymphatic filariasis: systematic review. *Tropical Medicine and International Health.* 10:818-825.

Cruz e Silva, J.A. (1971). Contribuição para o estudo dos helmintes parasitas vertebrados de Moçambique. *Memorial da Junta de Investigações do Ultramar*. 61:410–411.

Cruz, I., Cruz, M. E., Carrasco, F., & Horton, J. (1995). Neurocysticercosis: optimal dose treatment with albendazole. Journal of the Neurological Sciences.133: 152-154.

Dakkak, A. (1984.) Physiopathologie digestive des trichostrongylidoses ovines. Revue Médicine Veterinaire. 135:57-365.

Daoud, I. S., Herbert, I. V. (1982). Isolation of two lipoprotein antigens from the metacestodes of Taenia hydatigena (Pallas, 1766) and Taenia multiceps (Leske, 1780) and their evaluation in sero-diagnosis. *Veterinary Parasitology*. 11:155-64.

Dash, K.M. (1973). The life cycle of Oesophagostomum columbianum (Curtice, 1890) in Sheep. *International Journal for Parasitology*. 3:843-851.

Daoud, I. S., & Herbert, I. V. (1982). Isolation of two lipoprotein antigens from the metacestodes of Taenia hydatigena (Pallas, 1766) and Taenia multiceps (Leske, 1780) and their evaluation in sero-diagnosis. *Veterinary Parasitology*. 11:155-164.

Davis A, Dixon H, Pawloski Z. S. (1989). Multicentre clinical trials of benzimidazole-carbamates in human cystic echinococcosis (phase 2). *Bulletin of World Health Organization*. 67:503-8.

Dayan, A. D. (2003). Albendazole, mebendazole and praziquantel. Review of non toxicity and pharmacokinetics. *Acta Tropica*. 86:141-159.

Debackere, M., Landuyt, J., Vercruysse, J., McKellar, Q. (1993). The influence of Ostertagia circumcincta and Trichostrongylus colubriformis infections on the pharmacokinetics of febantel in lambs. *Journal of Veterinary Pharmacology and Therapeutics*. 16:261-274.

Del Brutto O. H, Roos K. L, Coffey C. S, Garcia H. H. (2006). Meta-analysis: Cysticidal drugs for neurocysticercosis: albendazole and praziquantel. *Annals of Internal Medicine*. 145:43–51.

Benoit, E., Besse, S., & Delatour, P. (1992). Effect of repeated doses of albendazole on enantiomerism of its sulfoxide metabolite in goats. American journal of Veterinary Research. 53: 1663-1665.

Delatour, P., Benoit, E., Bessé, S., Boukraa, A. (1991b). Comparative enantioselectivity in the sulfoxidation of albendazole in man, dogs and rats. Xenobiotica. 21:217-221.

Delatour, P., Benoit, E., Lechenet, J., Bessé, S. (1990). Pharmacokinetic in sheep and cattle of albendazole administered by an intraruminal slow release capsule. *Research in Veterinary Science*. 48:271-275.

Delatour, P., Garnier, F., Benoit, E., Caude, I. (1991a). Chiral behaviour of the metabolite albendazole sulphoxide in sheep, goats and cattle. Research in Veterinary Science. 50:134-138.

Deressa A., Tilahun T., Tadesse A., Beyene M., Gebrewold G., Pal M. (2012). Assessment of Coenurus cerebralis and its economic impact in sheep brain harvested at Ethiopian Health and Nutrition Research Institute, Ethiopia. International Journal of Livestock Research. 2: 217-226

Doganay, A., BIYIKOĞLU, G., Oce, H. (1999). Serodiagnosis of Coenurosis by ELISA in Experimental Infected Lambs. *Acta Parasitologica Turcica*. 23:185-189.

Doherty, M. L., McAllister, H., Healy, A. (1989). Ultrasound as an aid to Coenurus cerebralis cyst localisation in a lamb. *Veterinary Record*. 124:591.

Dorny, P., Brandt, J., Zoli, A., Geerts, S. (2003). Immunodiagnostic tools for human and porcine cysticercosis. *Acta Tropica*. 87:79-86.

Douch, P. G., Buchanan, L. L. (1979). Some properties of the sulphoxidase and sulphoxide reductases of the cestodes Moniezia expansa, the nematode Ascaris suum and mouse liver. *Xenobiotica.* 9: 675-679.

Dupouy-Camet, J., Kociecka, W., Bruschi, F., Bolas-Fernandez, F., Pozio, E. (2002). Opinion on the diagnosis and treatment of human trichinellosis. *Expert Opinion Pharmacotherapeutics*. 3:1117-1130.

Dyson, D. A., Linklater, K. A. (1979). Problems in diagnosis of acute coenurosis in sheep. *Veterinary Record*. 104:528-529.

Edwards, G. T., Herbert, I. V. (1982). Observations on the course of Taenia multiceps infections in sheep: clinical signs and post-mortem findings. *British Veterinary Journal*. 138:489-500.

El Sinnary, K. A., Tageldin, M. H., al Sumry, H. S. (1999). Outbreak of coenurosis (Taenia species) in anglonubian goats in the Sultanate of Oman. *Veterinary Record*. 144:296-7.

Elitok, B., Elitok, O. M., Kabu, M. (2006). Field trial on comparative efficacy of four fasciolicides. *Veterinary Parasitology*. 13:279-285.

Elliot D.C. (1984). Tapeworm (Moniezia expansa) in Sheep: anthelmintic treatment studies to assess possible pathogenic effects and production loss in young infected animals in field. New Zealend Veterinary Journal. 32:158-188

EMEA, E. M. A. (1997). Albendazole summary report by CVMP. EMEA/MRL/247/97- Final. EMEA.

Evrard, B., Chiap, P., DeTullio, P., F., G., Piel, G., Van Hees, T., Crommen, J., Losson, B., Delattre, L. (2002). Oralbioavailability in sheep of albendazole from a suspension and form a solution contaning hydroxypropyl-β-cyclodextrin. *Journal of Controlled Release*. 85:45-50.

Fargetton, X., Galtier, P., Delatour, P. (1986). Sulfoxidation of albendazole by a cytochrome P450-independent monoxygenase from rat liver microsomes. *Veterinary Research Communication*. 10:317-324.

Fleury, A., Bouteille, B., Garcia, E., Marquez, C., Preux, P. M., Escobedo, F., Sotelo, J., Dumas, M. (2001). Neurocysticercosis: validity of ELISA after storage of whole blood and cerebrospinal fluid on paper. *Tropical Medicine International Health*. 6:688-693.

Foreyt, W. J. (1988). Efficay and safety of albendazole against experimental induced Fasciola hepatica infections in goats. *Veterinary Parasitology*. 26:261-264.

Gall, C. (1996). Goat breeds of the world. CTA-Margraf Verlag, Germany.

Galtier, P. (1991). Métabolisme des benzimidazoles et activité inductrice enzymatique. *Revue Médicine Veterinaire.* 142:633-636.

Galtier, P., Alvineria, M., Steimer, J. L., Francheteau, P., Plusquellec, Y., Houin, G. (1991). Simultaneous pharmacokinetic modeling of drug and two metabolites: application to albendazole in sheep. *Journal Phamacology Science*. 80:3-10.

Galtier, P., Larrieu, G., Tufenkji, A., Franc, M. (1986). Incidence of experimetal fsciolosis on the activity of drug-metabolizing enzymes in lamb liver. *Drug Metabolism and Disposition*. 14:137-141.

Gandhi, M., Aweeka, F., Greenblat, R. M., Blaschke, T. F. (2004). Sex differences in pharmacokinetics and pharmacodynamics. *Annual Review Pharmacology and Toxicology*. 44:499-523.

Garcia, H. H., Moro, P. L., Schantz, P. M. (2007). Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fascioliasis. *Current Opinion in Infectious Disease*. 20:489-94.

Gasser, R. B., Chilton, N. B. (1995). Characterisation of taeniid cestode species by PCR-RFLP of ITS2 ribosomal DNA. *Acta Tropica*. 59:31-40.

Gasser, R. B., Zhu, X., McManus, D. P. (1999b). NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit I sequences compared for members of the genus Taenia (Cestoda). International *Journal for Parasitology*. 29:1965-70.

Gasser, R. B., Zhu, X., Woods, W. (1999a). Genotyping Taenia tapeworms by single-strand conformation polymorphism of mitochondrial DNA. *Electrophoresis*. 20:2834-2837.

Gauci, C., Vural, V., Oncel, T., Varcasia, A., Daniam, V., Kyngdon, C.T., Craig, P.S. Anderson, G.A., Lightowlers, M.W. (2008). Vaccination with recombinant oncosphere antigens reduces the susceptibility of sheep to infection with *Taenia multiceps*. *International Journal for Parasitology*. 38:1041-1050.

Ghazaei, C. (2007). Evaluation therapeutic effects of antihelminthic agents albendazole, fenbendazole and praziquantel against coenurosis in sheep. *Small Ruminante Research*. 71:48-51.

Ghisi, M., Kaminsky, R., Maser, P. (2007). Phenotyping and genotyping of Haemonchus contortus isolates reveals a new putative candidate mutation for benzimidazole resistance in nematodes. Veterinary Parasitology. 144:313-320.

Gicik, Y., Kara, M., & Arslan, M. O. (2007). Prevalence of Coenurus cerebralis in sheep in Kars Province, Turkey. BULLETIN-VETERINARY INSTITUTE IN PULAWY.51: 379.

Godara, R., Borah, M. K., Sharma, R. L., & Jangir, B. L. (2011). Caprine coenurosis with special reference to hepatic coenurosis. Comparative Clinical Pathology. 20: 277-280.

Gokbulut, C., Akar, F., McKellar, Q. A. (2006b). Plasma disposition and faecal excretion of oxfendazole, febendazole and albendazole following oral administration to donkeys. The Veterinary Journal. 172:166-172.

Gokbulut, C., Bilgili, A., Hanedan, B., McKellar, Q. A. (2007) Comparative plasma disposition of fenbendazole, oxfendazole and albendazole in dogs. *Veterinary Parasitology.* 148:279-287

Gokbulut, C., Cirak, V. Y., Senlik, B. (2006a). Plasma disposition and faecal excretion of netobimin metabolites and enantiospecific disposition of alendazole sulphoxide produced in ewes. *Veterinary Research Communication*. 30:791-805.

Gokbulut, C., Cirak, V.Y., Senlik, B., Yildirim, F., McKellar, Q.A. (2009). Pharmacological assessment of netobimin as a potential anthelmintic for use in horses: Plasma disposition, faecal excretion and efficacy. *Research in Veterinary Science*. 86:514-520.

Gonzalez, L. M., Ferrer, E., Spickett, A., Michael, L. M., Vatta, A. F., Garate, T., Harrison, L. J., Parkhouse, R. M. (2007). The Taenia saginata homologue of the major surface antigen of Echinococcus spp. is immunogenic and 97% identical to its *Taenia solium* homologue. *Parasitology Research*. 101:1541-9.

Gonzalo-Orden., J. M., Díez, A., Altónaga, J. R., Gonzalo, J. M., Orden, M. A. (1999). Computed Tomographic Findings in Ovine Coenurosis. *Veterinary Radiology & Ultrasound*. 40:441-444.

Gosttstein, B., Tsang, V. C., Schantz, P. M. (1986). Demonstration of specific and cross-reactive components of Taenia solium metacestodes. *American Journal of Tropical Medicine and Hygiene.* 35:308-313

García, H. H., González, A. E., Del Brutto, O. H., Tsang, V. C., Llanos-Zavalaga, F., Gonzalvez, G., ... & Cysticercosis Working Group in Peru. (2007). Strategies for the elimination of taeniasis/cysticercosis. *Journal of the Neurological Sciences*. 262: 153-157.

Gottschall, D. W., Theodorides, V. J., Wang, R. (1990). The metabolism of benzimidazole anthelmintics. *Parasitology Today*. 6:115-124.

Gracia, H., Gonzalez A.E., Evans, C.A.W., Gilman, R.H. (2003). *Taenia solium* cysticercosis. *Lancet*. 362:547-556.

Gracia, J. L., Bolás, F., Torrado, J. J. (2003). Bioavailability and efficacy charactristics of two different oral liquid formulation of albendazole. *International Journal for Parasitology*. 250:351-358.

Gyruik, R. J., Chow, A. W., Zaber, B., Brunner, E. I., Miller, J. A., Villani, A. J., Petra, L. A., Parish, R. C. (1981). The metabolism of albendazole in cattle, sheep, rats and mice. *Drug Metabolism and Disposition.* 9:503-508.

Hago, B. E. D., Abu-Samara, M. T. (1980). A case of Multiceps gaigeri Coenurosis in Goat. *Veterinary Record.* 7:191-194.

Hanjeet, K., Mathias, R.G. (1992). The efficacy of treatment with albendazole. *Acta Tropica*. 50:111-114.

Hansen J, Perry B. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants. *International Laboratory for Research on Animal Diseases*. Nairobi, Kenya

Haridy, M., Sadan, M., Omar, M., Sakai, H., & Yanai, T. (2014). Coenurus cerebralis cyst in the orbit of a ewe: research communication. Onderstepoort Journal of Veterinary Research.81: 1-4.

Harper, C.K., Penzhorn B.L. (1999). Ocorrence and diversity of Coccidia in indigenous Saanen and crossbred goats in South Africa. Veterinary Parasitology. 82:1-9.

Helle, O. (1986). The efficacy of fenbendazole and albendazole against the lungworm Muellerius capillaris in goats. Veterinary Parasitology. 22:293-301.

Hennessy, D. R. (1993). Pharmacokinetic disposition of benzimidazole drugs in the ruminant gastrointestinal tract. Parasitology Today. 9:329-333.

Hennessy, D. R., Prichard, R. K. (1981). The role of absorbed drug in the efficacy of oxfendazole against gastrointestinal nematodes. *Veterinary Research Communication*. 5:45-49.

Hennessy, D. R., Sangster, N. C., Steel, J. W., Collins, G. H. (1993b). Comparative pharmacokinetic behaviour of albendazole in sheep and goats. *International Journal for Parasitology*. 23:321-325.

Hennessy, D. R., Steel, J. W., Lacey, E., Eagleson, G. K., Prichard, R. K. (1989). The disposition of albendazole in sheep. *Journal of Veterinary Pharmacology and Therapeutics*. 12:421-429.

Hennessy, D., Sangster, N. C., Steel, J., Collins, G. H. (1993a) Comparative kinetic disposition of oxfendazole in sheep and goats before and during infection with Haemonchus contortus and Trichostrongylus colubriformis. *Journal of Veterinary Pharmacology and Therapeutics*. 16:245-253.

Horton, J. (2003). Albendazole for the treatment of echinococcosis. Blackwell Publishing Fundamental & *Clinical Pharmacology*. 17:205-212.

Horton, R. J. (1990). Benzimidazole in a wormy world. *Parasitology Today*. 6:106.

Ibechukwu, B. I., & Onwukeme, K. E. (1991). Intraocular coenurosis: a case report. British journal of ophthalmology. 75: 430-431.

Hoste, H., Torres-Acosta, J. F., Paolini, V., Aguilar-Caballero, A., Etter, E., Lefrileux, Y., Broqua, C. (2005). Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. Small Ruminant Research, 60: 141-151.

INE. (2011). Censo Agro-Pecário 2009-2010: Resultados Preliminares – Moçambique. Instituto Nacional de Estatisticas. p77.

Ing, M. B., Schantz, P. M., Turner, J. A. (1998) Human coenurosis in North America: case reports and review. *Clinical Infectious Disease*. 27:519-23.

Ingold, K., Bigler, P., Thormann, W., Cavaliero, T., Gottstein, B. Hemphill, A. (1999). Efficacies of Albendazole Sulfoxide and Albendazole Sulfone against In Vitro-Cultivated Echinococcus multilocularis Metacestodes. *Antimicrobial Agents and Chemotherapy*. 43:1052–1061.

Ioannidou, E., Psalla, D., Papadoupoulos, Diakou, A., Papanikolapoulou, V., Karatzias, H., Palizopoulou, Z., Giadinis, N. (2015). Regurgitations in a Lamb with Acute Coenurosis – A case Reprot. *Iran Journal for Parasitology.* 10: 301 -305

Ito, A., Plancarte, A., Nakao, M., Nakaya, K., Ikejima, T., Piao, Z. X., ... & Margono, S. S. (1999). ELISA and immunoblot using purified glycoproteins for serodiagnosis of cysticercosisin pigs naturally infected with Taenia solium. Journal of Helminthology. 73: 363-365.

Islam, S., Kalita, D., Bhuyan, D., Rahman, T., & Saleque, A. (2006). Ocular coenurosis in goat. Journal of Veterinary Parasitology. 20: 53-55.

Jalila, A., Dorny, P.,Sani, R., Salin, N.B., Vercruysse, J. (1998). Coccidial infections of goats in Selangor peninsular Malaysia. *Veterinary Parasitology*. 74:165-172.

Jamshidi, M, Mohraz, M., Zangeneh, M., Jamshidi A. (2008). The effect of combination therapy with albendazoleand praziquantel on hydatid cyst treatment. *Parasitology Research*. 103:195-19.

Jemili, M. H., Galtier, P., Dorchis, P., Benromdhane, S., Kilani, M. (1994). Triclabendazole effect on Fasciola-hepatica - induced decrease of biotrnasformation liver-enzymes. *Revue Médicine Veterinaire*. 145:349-353.

Jeon, H.K.,Eom, K.S. (2007). *Taenia asiatica* and Taenia saginata: genetic divergence estimated from their mitochondrial genomes. *Experimental Parasitology*. 113:758-61.

Jiménez-González, A., De Armas-Serra, C., Criado-Fornelio, A., Casada-Escribano, N., Rodríguez-Caabeira, F., Diéz, J. C. (1991). Preliminar characterization and interaction of tubulin from Trichichinella spiralis larvae with benzimidazole derivatives. *Veterinary Parasitology*. 39:89-99.

Kagira, J., & Kanyari, P. W. N. (2001). The role of parasitic diseases as causes of mortality in small ruminants in a high-potential farming area in central Kenya. Journal of the South African Veterinary Association. 72: p-150.

Kaufman, J. (1996). Parasitic Infection of Domestic Animals. A diagnostic Manual. Birkhauser Verlag., Berlin, Germany

Kern, P. (2006). Medical treatment of echinococcosis under the guidance of Good Clinical Practice (GCO/ICH). Parasitology International. 55:S273-S282.

Kheirandish, R., Sami, M., Azizi, S. & Mirzaei, M. (2012). Prevalence, predilection sites and pathological findings of Taenia multiceps coenuri in slaughtered goats from south-east Iran. Onderstepoort Journal of Veterinary Research. 79:Art. #436, 5 pages. http:// dx.doi.org/10.4102/ojvr. v79i1.436

Khurana, N., Garg, R. K., Verma, R., Malhotra, H. S., Singh, M. K., & Shukla, R. (2012). Three-day versus 15-day course of albendazole therapy in solitary cysticercus granuloma: An open label randomized trial. Journal of the Neurological Sciences. 316: 36-41.

Kimberling, C. V. (1988). Jansen and Swift's Diseases of Sheep Lea & Febiger, Phladelphia, USA.

Kish, G. F., Khodakaram-Tafti, A., Hajimohammadi, A., & Ahmadi, N. (2013). Clinical and morphopathological characteristics of an enzootic occurrence of acute coenurosis (Coenurus cerebralis) in a sheep herd. Journal of Parasitic Diseases. 1-4.

Kohri, N., Yamayoshi, Y., Xin, H., Iseki, K., Sato, N., Todo, S., Miyazaki, K. (1999). Improving the oral bioavailability of albendazole in rabbits by the solid dispersion technique. Journal Pharmaceutic and Pharmacology. 51:159-164.

Koumnenou, A., Argyroudis, S., Giadinis, N., Dessiris, A. (2000). Surgical treatment of coenurosis (gid) in sheep. *Veterinary Record*. 147:242-244.

Kusiluka, L.J.M., Kambarage, D.M., Matthewman, R.W., Harrison, L.J.S., Daborn, C.J. (1996). Coccidiosis in small ruminants in Tanzania. *Small Ruminant Research*. 21:127-131.

Lacey, E. (1990). Mode of action of benzimidazoles. *Parasitology Today*. 6:112-115.

Laemmli, U. K. (1970).Cleavage of structural proteins during the aasembly of the head of bacteriophage T4. *Nature* 227: 680-685

Lamka, J., Krizová, V., Cvilink, V., Savlik, M., Velik, J., Duchacek, L., Szotakova, B., Skolova, L. (2007). A single adulticide dose of albendazole induces cytochromes P4501A in mouflon (Ovis musimon) with dicroceliosis. *Veterinari Medicina*. 52:343-352.

Lange, H., Eggers, R., Bricher, J. (1988). The increse systemic bioavailability of albendazole when taken with a fatty meal. *European Journal of Clinical Pharmacology*. 34:315-317.

Lanusse C., Virkel G., Sánchez S., Alvarez, L.I., Lifschitz, A.I., Imperiale, F., Monfrinotti, A. (1998). Ricobendazole kinetics and availability following subcutaneous administration of a novel injectable formulation to calves. *Research in Veterinary Science*. 65: 5-10.

Lanusse, C. E., Gascon, L. H., Prichard, R. K. (1995). Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfndazole and their metabolites in adult sheep. *Journal of Veterinary Pharmacology and Therapeutics*. 18:196-203.

Lanusse, C. E., Nare, B., Gascon, L. H., Prichard, R. K. (1992a). Bioconversion of netobimin pro-drug by gastrointestinal fluids of ruminants. *European Journal of Drug Metabolism and Pharmacokinetic*. 17:121-128.

Lanusse, C. E., Nare, B., Gascon, L. H., Prichard, R. K. (1992b). Metabolism of albendazole and albendazole sulphoxide by ruminal and intestinal fluids of sheep and cattle. *Xenobiotica*. 22:419-426.

Lanusse, C., Gascon, L., Prichard, R. (1993). Gastrointestinal distribution of albendazole metabolites following netobimin administration to cattle: relationship with plasma disposition kinetics. *Journal of Veterinary Pharmacology and Therapeutics*. 13:38-47.

Lanusse, C.E.,Prichard, R.K. (1993). Relationship between pharmacological properties and clinical efficacy of ruminant anthelmintics. *Veterinary Parasitology*. 49:123-158.

Lavikainen A., Haukisalmi V., Lehtinen M.J., Henttonen H., Oksanen A., Meri S.(2008). A phylogeny of members of the family Taeniidae based on the mitochondrial *cox*1 and *nad*1 gene data. *Parasitology*. 135:1457–1467

Le Riche, P. D., Sewell, M. M. H. (1978). Differentiation of Taeniid cestodes by enzyme electrophoresis. *International Journal for Parasitology*. 8:479-483.

Li W.H., Jia W.Z., QuZ.G., Xie Z.Z., Luo J.X., Yin H., SunX.L., Blaga R. and FuB.G. (2013). Molecular Characterization of *Taenia multiceps* Isolates from Gansu Province, China by Sequencing of Mitochondrial Cytochrome C Oxidase Subunit 1. *The Korean Journal of Parasitology*. 51: 197- 201

Li, W.H., Qu, Z.G., Zhang, N.Z., Yue, L., Jia, W.Z., Luo, J.X., Yin, H., Fu, B.Q. (2015). Molecular characterization of enolase gene from *Taenia multiceps*. *Research in Veterinary Science*. 102:53 -58.

Li, T., Quiao, G. L., Hu, G. Z., Meng, F. D., Qui, Y. S., Zhang, X. Y., Guo, W. X., Yie, H. L., Li, S. Y. (1995). Comparative plasma and tissue pharmacokinetics and drug residue profiles of different chemotherapeutants in fowls and rabbits. *Journal of Veterinary Pharmacology and Therapeutics*. 18:260-273.

Lifschitz, A., Virkel, G., Mastronmarino, M., Lanusse, C. (1997). Enhanced plasma availability of the metabolites of albendazole in fasted adult sheep. *Veterinary Research Communications*. 21:201-211.

Lightowlers, M. W., Mitchell, G. F., Bowtell, D. D. L., Anders, R. F., Rickard, M. D. (1984). Immunization against Taenia taeniaeformis in mice: Studies on the characterization of antigens from. *International Journal for Parasitology*. 14: 321-333

Lightowlers, M. W., Rolfe, R., Gauci, C. G. (1996). Taenia saginata: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. *Experimental Parasitology*. 84:330-8.

Ligios, C., Vigliette, A., Carta, P., Dexter, G., Agrimi, U., Simmons, M. M. (2004). Cinicopathological findings in Sheep from Sardinia showing Neurological Signs of Disease. *Veterinary Record*. 154:365-370.

Liu, L. X., Weller, P.F.. (1996). Antiparasitic drugs. The New England Journal of Medicine. 334:1178–1184.

Madhu, D. N., Mahan, T., Sudhakar, N. R., S Maurya, P., Banerjee, P. S., Sahu, S., & Pawde, A. M. (2014). Coenurus gaigeri cyst in the thigh of a goat and its successful management. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology, 38: 286–288.

Maichomo M.W., Kaira J.M., Walter T. (2004). The point prevalence of gastro-intestinal parasites in calves, sheep and goats in Magadi division, south-western Kenya. Onderstepoort Journal of Veterinary Research. 71:257–261

Mariaux J. (1996). Cestode Systematics: Any Progress?. *International Journal for Parasitology*. 26: 234 – 243.

Markoski, M. M., Trindade, E. S., Cabrera, G., Laschuk, A., Galanti, N., Zaha, A.,Ferreira, H. B. (2006). Praziquantel and albendazole damaging action on in vitro developing Mesocestoides corti (Platyhelminthes: Cestoda). *Parasitology International*. 55(1), 51-61.

Marriner, S. E., Bogan, J. A. (1980). Pharmacokinetic of albendazole in sheep. American Journal of *Veterinary Research*. 41:1126-1129.

Marriner, S. E., Evans, E. S., Bogan, J. A. (1985). Effect of parasitism with Ostertagia circumcincta on pharmacokinetics of febendazole in sheep. *Veterinary Parasitology*. 17:239-249.

Martin, R. J. (1997) Modes of action of anthelmintic drugs. *The Veterinary Journal*. 154:11-34.

Matthaiou, D.K., Panos, G., Adamidi, E.S., Falagas, M.E. (2008). Albendazole versus Praziquantel in the treatment of neurocysticercosis: A meta-analysis of camparative trials. *Plos Negletec Tropical Diseases*. 2, e194, 1-7.

Mayta, H., Hancock, K., Levine, M. Z., Gilman, R. H., Farfan, M. J., Verastegui, M., Lane, W. S., Garcia, H. H., Gonzalez, A. E., Tsang, V. C. (2007). Characterization of a novel Taenia solium oncosphere antigen. *Molecular and Biochemical Parasitology*. 156:154-61.

Mbuh, J. V., Ndamukong, K. J. N., Ntonifor, N., & Nforlem, G. F. (2008). Parasites of sheep and goats and their prevalence in Bokova, a rural area of Buea Sub Division, Cameroon. Veterinary Parasitology. 156: 350-352.

McKellar, Q. A., Scott, E. W. (1990). The benzimidazole anthelmintic agents- a review. *Journal of Veterinary Pharmacology and Therapeutics*. 13:223-247.

McKellar, Q., Coop, R. L., Jackson, F. (1995). The pharmacokinetics of albendazole metabolites following administration of albendazole, albendazole sulphoxide and netobimin to one-month and eight-months - old sheep. *International Journal for Parasitology*. 10:1207-1212.

McKellar, Q., Jackson, F., Coop, L., Baggot, J. D. (1993). Plasma profiles of albnedazole metabolites after administration of netobimin and albendazole in sheep: effects of parasitism and age. *British Veterinary Journal.* 149: 101-113.

McKellar, Q., Jackson, F., Coop, L., Jackson, E., Scott, E. (1991) Effect of parasitism with Nematodirus battus on the pharmacokinetics of levamizole, ivermectin and netobimin. *Veterinary Parasitology*. 39:123-136.

McManus D.P. (2005). Molecular discrimination of taeniidcestodes. *Parasitology International.* 55:31–37

Medina, M. T., Genton, P., Montoya, M. C., Cordova, S., Dravet, C., & Sotelo, J. (1993). Effect of anticysticercal treatment on the prognosis of epilepsy in neurocysticercosis: a pilot trial. Epilepsia. 34:1024-1027.

Meneses da Silva, (2003). Hydatid cyst of the liver - criteria for the selection of appropriate treatment. *Acta Tropica*. 85:237–242.

Merino, G., Molina, A. J., Garcia, J. L., Pulido, M. M., Prieto, J. G., Alvarez, A. I. (2003b). Intestinal elimination of albendazole sulfoxide: pharmacokinetic effects of inhibitors. *International Journal of Pharmaceutics*. 263:123-32.

Merino, G., Molina, A. J., García, J. L., Pulido, M. M., Prieto, J. G., Alvarez, A. I. (2003a). Ginseng increases intestinal elimination of albendazole sulfoxide in rat. *Comparative Biochemistry and Physiology Part C.* 136:9-15.

Mingjie, W., Shuhua, X., Junjie, C., Bin, L., Cheng, F., Weixia, S., Hotez, P. (2002). Albendazole-soybean oil emulsion for the treatment of human cystic echinococcosis: evaluation of bioavailability and bioequivalence. Acta Tropica. 83:177-181.

Miran M.B., Nzalawahe J., Kassuku A.A., Swai E.S., (2015). Prevalence of coenurosis in sheep and goats at three slaughter slabs in Ngorongoro District, Tanzania. Tropical Animal Health and Production. DOI 10.1007/s11250-015-0903-7.

Mirfazaelian, A., Dadashzadeh, S., & Rouini, M. R. (2002). A high performance liquid chromatography method for simultaneous determination of albendazole metabolites in human serum. Journal of Pharmaceutical and Biomedical Analysis. 30: 1249-1254.

Monsang, S. W., Singh, M. N., Kumar, M., Roy, J., Pal, S. K., & Singh, W. R. (2014). Diagnosis and surgical management of non-cerebral coenurosis (Taenia gaigeri) in local Black Bengal goat of Tripura: report of six cases. Journal of Parasitic Diseases. 1-5.

Moreno, L. Echevarria, F., Muñoz, F., Alvarez, L., Sanches Bruni, S., Lanusse, C. (2004). Dose-dependent activity of albendazole against benzimidazole-resistant nematodes in sheep: relationship between pharmacokinetics and efficacy. Experimental Parasitology. 106:150-157.

Morgado, F.P. (2000). A pecuária no Norte de Moçambique. Vega: Portugal.

Moroni, P., Buronfose, T., Longin-Sauvageon, C., Delatour, P., Benoit, E. (1995). Chiral sulfoxidation of albendazole by te flavin adenine dinucleotide-containing and cytochrome P450- dependent monoxygenases from rat liver microsomes. *Drug Metabolism and Disposition*. 23:106-165.

Naghashyan, H. Z., Hartunyan, H. G. (2001). Postsurgical Differentiation of Coenurosis and Echinococcosis. *Online Journal of Veterinary Research*. 5:162-165.

Nagy, J., Schipper, H. G., Koopmans, R. P., Butter, J. J., van Boxtel, J., Kager, P. A. (2002). Effect of grapefruit juice or cimetidine coadministration on albendazole bioavailability. *American Journal of Tropical Medicine and Hygiene.* 66:260-263.

Nakao, M., Sako, Y., Yokoyama, N., Fukunaga, M., Ito, A. (2000). Mitochondrial genetic code in cestodes. *Molecular Biochemistry Parasitology*.111: 415-424.

Nakao,M., Lavikainen,A., Iwaki,T., Haukisalmi,V., Konyaev,S., Oku,Y., Okamoto,M. and Ito,A. (2013). Molecular phylogeny of the genus Taenia (Cestoda: Taeniidae):Proposals for the

resurrection of Hydatigera Lamarck, 1816 and the creation of a new genus Versteria. *International for Parasitology*. 43: 427-437

Navarro, M., Cristofol, C., Carretero, A., Arboix, M. & Ruberte, J. (1998) Anthelmintic induced congenital malformations in sheep embryos using netobimin. Veterinary Record. 4: 86 - 90.

Negita, T., Ito A. (1994). In vitro hatching of oncospheres of Taenia taeniaeformis using eggs isolated from fresh, frozen, formalin fixed and ethanol-fixed segments. Journal of Helminthology. 68: 271- 272.

Ngomuo, A. J., Marriner, S. E., Bogan, J. A. (1984). The pharmacokinetics of fenbendazole and oxfendazole in cattle. *Veterinary Research Communication*. 8:187-193.

Nooruddin, M., Dey, A. S., Ali, M. A. (1996). Coenurosis in Bengal goats of Bangladesh. *Small Ruminant Research*. 19:77-81.

Nourani, H., & Kheirabadi, K. P. (2009). Cerebral coenurosis in a goat: pathological findings and literature review. Comparative Clinical Pathology. 18: 85-87.

Nwosu, L.O., Okon, E.D., Chiejina, S.N., Igboke, I.O., Mbaya, A.W., Columbus, P.K. Chagwa, L.L., Daniel-Igwe, G. (2013). Comparative Clinical Pathology. 22:605-610.

Oge, H., Oge, S., Gonenc, B., Ozbakis, G., & Asti, C. (2012). Coenurosis in the lumbar region of a goat: a case report. Vet Med-Czech, 57: 308-313.

Olanike, A. O., Olayide, A. J., Oludunsin, F. O., Racheal, A. O., & Japhet, D. W. (2015). Prevalence of Gastrointestinal Parasites of Goats in Ibadan, Southwest, Nigeria. World Journal of Agricultural Research. 3: 49-51.

Olsen, A., Namwanje, H., Nejsum, P., roepstorff, A., Thamsborg, S.M. (2009). Albendazole and mebendazole have low efficacy against Trichuris trichiura in school-age children in Kabale District, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 103:443-446.

Olson P.D. and Tkach V.V. (2005). Advances and Trends in the Molecular Systematics of the ParasiticPlatyhelminthes. Advances in Parasitology. 60:165:243

Onar, E., (1990). Efficacy of thiophanate and albendazole against natural infections of Dicrocoelium dentriticum, Fasciola hepatica, and gastrointestinal nematodes and cestodes in sheep. Veterinary Parasitology. 35:139-145.

Opatrny L., Prichard, R., Snell, L., Maclean, D. (2005). Death related to albendazole-induced pancytopenia: Case report and review. *American Journal of Tropical Medicine and Hygiene*. 72:291–294.

Oriol, C., Oriol, R. (1975). Physochemical proprrties of lipoprotein antigens of Echinococcus granulosus. *American Journal of Tropical Medicine and Hygiene*. 24:96-100.

Ortona, E., Riganò, R., Buttari, B., Delanardo, F., Ioppolo, S., Margutti, P., Profumo, E., Teggi, A., Vaccari, S., Siracusano, A. (2003). An update on immunodiagnosis of cystic echinococcosis. *Acta Tropica*. 85:165-171.

Oryan, A., Moghaddar, N., Gaur, S. N. (1994). Metacestodes of sheep with special reference to their epidemiological status, pathogenesis and economic implications in Fars Province, Iranian. *Veterinary Parasitology*. 51:231-40.

Oryan, A., Nazifi, S., Sharifiyazdi, H., Ahmadnia, S. (2010). Pathological, molecular, and biochemical characterization of *Coenurus gaigeri* in Iranian native goats. *Journal of Parasitology*. 96:961–967.

Osman, A. M. M., Jacobs, D. E., Fisher, M. A., Pilkington, J. G., & Jones, P. A. (1996). Effect of sub-lethal doses of albendazole on the fecundity of Dictyocaulus viviparus. Research in veterinary science. 61: 92-93.

Oukessou, M., Chokounda, S. (1997). Effect of diet variations on the kinetic disposition of oxfendazole in sheep. International Journal for Parasitology. 27:1347-1351.

Owhoeli, O., Elele, K., & Gboeloh, L. B. (2014). Prevalence of Gastrointestinal Helminths in Exotic and Indigenous Goats Slaughtered in Selected Abattoirs in Port Harcourt, South-South, Nigeria. Chinese Journal of Biology. 2014.

Oxberry, M.E., Reynoldson, J.A., Thompson, R.C.A. (2000). The binding and distribution of albendazole and its principal metabolitesin Giardia duodenalis. *Journal of Veterinary Pharmacology and Therapeutics*. 23:113–120.

Oxberry, M. E., Geary, T. G., Winterrowd, C. A., Prichard, R. (2001). Individual expression of recombinant α- and β- tubulin from Haemonchus contortus: polymerization and drug effects. *Protein Expression and Purification*. 21:30-39.

Ozmen, O., Sahinduran, S., Haligur, M., Sezer, K. (2005). Clinicopathologic observations on Coenurus cerebralis in naturally infected sheep. *Schweiz Arch Tierheilkd*. 147:129-34.

Palomares, F., Palencia, G., Ambrosio, J. R., Ortiz, A., Jung-Cook, H. (2006). Evaluation of the efficacy of albendazole sulphoxide and praziquantel in combination on Taenia crassiceps cysts: in vitro studies. *Journal of Antimicrobial Chemotherapy*. 57:482-488.

Palomares, F., Palencia, G., Pérez, R., González-Esquivel, D., Castro, N., Cook, H. J. (2004). In vitro effects of albendazole sulfoxide and praziquantel against Taenia solium and Taenia crassiceps cysts. Antimicrobial Agents and Chemotherapy. 48:2302-2304.

Pandey, V. S., Ndao, M., & Kumar, V. (1994). Seasonal prevalence of gastrointestinal nematodes in communal land goats from the highveld of Zimbabwe. Veterinary Parasitology. 51: 241-248.

Pathak, A. K., & Pal, S. (2008). Seasonal prevalence of gastrointestinal parasites in goats from Durg district of Chhattisgarh. Veterinary World. 1: 136-137.

Penicaut, B., Maugein, P., Maisonneuve, H., Rossignol, J. F. (1983). Pharmacokinetics and urinary metabolism of albendazole in man. *Bulletin de la Societe de Pathologie Exotique et des Filiales*. 76:698-708.

Peniche-Cardeña, A., Dominguez-Alpizar J.L., Sima-Alvarez, R., Argaez-Rodriguez, F., Fraser, A.,Craig, P.S., Rodriguez-Canul R. (2002). Chemotherapy of porcine cysticercosis with albendazole sulphoxid. *Veterinary Parasitology*. 108:63-73A.

Piel, G., Evrard, B., Van Hees, T., Llabres, G., Delattre, L. (1999). Development of a parenteral and of an oral formulation of albendazole with cyclodextrins. *Pharmaceutical Science.* 9:257-260.

Popesko, P., Hájovska, B., Komárek, V., Marvan, F., Vrzgulova, M. (1992). Príroda, Bratislava, Czechoslovakia.

Popoesko, P. (1984). Atlas of Topographical Anatomy of the Domestic Animals. W.B. Saunders Company, Philadelphia, London, Toronto.

Price, T. C., Dresden, M. H., Alvarado, T., Flangan, J., Chappell, C. L. (1989). Coenuriasis in a Spectacled Langur (Presbytis obscura): Praziquantel Treatment and the Antibody Response to Cyst Antigens. *American Journal of Tropical Medicine and Hygiene*. 40:514-520.

Prichard, R., Hennessy, D., Steel, J., Lacey, E. (1985). Metabolic concentrations in plasma following treatment of cattle with five anthelmintics. *Research in Veterinary Science*. 39:173-178.

Radostits, O. M., Gay, C. C., Blood, D. C., Hinchcliff, K. W. (2000). Veterinary Medicine - A textbook of the diseases of cattle, sheep, pigs, goats and horses. Ninth Edition, W.B. Saunders, London, UK.

Redondo, P. A., Alvarez, A. I., Gracia, J. L., Larrodé, O. M., Merino, G., Prieto, J. G. (1999). Presystemic metabolism of albendazole: experimental evidence of an eflux process of albendazole sulphoxide to intestinal lumen. *Drug Metabolism and Disposition*. 27:736-740.

Rinaldi, L., Veneziano, V., & Cringoli, G. (2007). Dairy goat production and the importance of gastrointestinal strongyle parasitism. Transactions of the Royal Society of Tropical Medicine and Hygiene. 101: 745-746.

Robinson, M. W., McFerran, N., Trudgett, A., Hoey, L., Fairweather, I. (2004). Possible model of benzimidazole binding to β-tubulin disclosed by invoking an inter-domain movement. Journal of Molecular Graphics and Modelling. 23:275-284.

Rocha, M.A., Santos, J.M.S., Gomes, E.C.S., Rocha, M.A., Rocha C.F., Carvalho, G.T.C., Costa, B.S. (2008). Treatment of cerebral cysticercosis with albendazole in elevated dosages. *Arquivos de Neuropsiquiatria.* 66:114-116.

Rodrigues, J., Bories, C., Emery, I., Fessi, H., Devissaguet, J., Liance, M. (1995). Development of an injectable formulation of albendazole and in vivo evaluation of its efficacy against Echinococcus multilocularis metacestode. *International Journal for Parasitology*. 25:1437-1441.

Rolin, S., Souhaili-El Amri, H., Batt, A.-M., Lavy, M., Bagrel, D., Siest, G. (1989). Study of the in vitro bioactivation of albendazole in human liver microsomes and hepatoma cell lines. *Cell Biology and Toxicology*. 5:1-14.

Rostami S., Salavati R., Beech R.N., Sharbatkhori M., Babaei Z., Saedi S., Harandi M.F. (2013). Cytochrome c oxidase subunit 1 and 12S ribosomal RNAcharacterization of *Coenurus cerebralis* from sheep in Iran. *Veterinary Parasitology*. 197:141 – 151.

Rruvalcaba, I.C.,Reveles-Hernández, G., Saldivar- elias, S., Muñoz-Escobedo, J. , Vallarta, M.M., Moreno-García, A., (2006). Evaluation of 3 antiparasites on intestinal and muscular phase infection of Trichinella spiralis in the pig model. Revista Electrónica de Veterinaria REDVET VII, Nº 06, http://www.veterinaria.org/revistas/redvet

Saeed, A.M.A, (2007). Efficacy of albendazole against experimental infection of Raillietina tetraqona in chickens. *Research Journal of Pharmacology*. 1:5-8.

Sánchez, S., Alvarez, A., Pis, A., Quiroga, M., Lanusse, C. (1999). Differences in plasma and abomasal kinetics of albendazole and its metabolites in calves grazed on pasture or fed a grain-based diet. *Research in Veterinary Science*. 66:223-230.

Sánchez, S., Alvarez, L., Lanusse, C. (1997). Fasting-induced changes to the pharmacokinetic behaviour of albendazole and its metabolites in calves. *Journal of Veterinary Pharmacology and Therapeutics*. 20:38-47.

Sánchez, S., Alvarez, L., Sallovitz, J., Lanusse, C. (2000). Enhanced plasma and target tissue availabilities of albendazole and albendazole sulphoxide in fasted calves: evaluation of different fasting intervals. *Journal of Veterinary Pharmacology and Therapeutics*. 23: 193-201.

Sangster, N. C., Rickard, J. M., Collins, G. H., Hennessy, D. R., Steel, J. W. (1992). Use of a controlled-release albendazole capsule in goats. *Australian Veterinary Journal*. 69:67-68.

Santos, H. T., Santos, A. F., & de la Rue, M. L. (2008). The action of albendazole on hydatid cysts in sheep experimentally infected with eggs of Echinococcus granulosus. Journal of Helminthology. 82: 109-112.

Sanyal, P. K. (1998a). The pharmacokinetics and efficacy of long-term low-level and split-dose administration of albendazoel through in-feed formulations against ovine and caprine parasitic gastroenteritis. Veterinary Research Communication. 22:467-477.

Sanyal, P. K. (1998b). Effect of single and divided dose administration on the pharmacokinetics of albendazole in sheep and goat. *The Veterinary Journal*. 155:311-316.

Scala, A., Cancedda, G. M., Varcasia, A., Ligios, C., Garippa, G., Genchi, C. (2007). A survey of Taenia multiceps coenurosis in Sardinian sheep. Veterinary Parasitology. 143:294-298.

Scala, A., Varcasia, A. (2006). Updates on morphobiology, epidemiology and molecular characterization of coenurosis in sheep. Parassitologia. 48:61-63.

Schipper, H. G., Koopmans, R. P., Nagy, J., Butter, J. J., Kager, P. A., van Boxtel, J. (2000). Effect of dose increase or cimedidine co-administration on albendazole bioavailability. American Journal of Tropical Medicine and Hygiene. 63:270-273.

Schuster, R. K., Sivakumar, S., & Wieckowsky, T. (2010). Non-cerebral coenurosis in goats. Parasitology research, 107: 721-726.

Scott, P. R. (2012). Diagnosis and treatment of coenurosis in sheep. Veterinary Parasitology, 189: 75-78.

Seckin, H., Yagmurlu, B., Yigitkanli, K., Kars, H. Z. (2008). Metabolic changes during successful medical therapy for brain hydatid cyst: case report. Surgical Neurology. 70:186-189.

Sharma D. K., Singh, Nem, Tiwari, H. A. (1998). Prevalence and pathology of coenurosis in organized goat farms. *Journal of Veterinary Parasitology*. 12:30-32

Sharmaa, D. K., Chauhanb, P. P. S. (2006). Coenurosis status in Afro-Asian region: A review. *Small Ruminant Research.* 64:197-202.

Shyale, S., Choadary, K. P. R., Krishniah, Y. S. R., Bhat, N. (2006). Pharmacokinetic evaluation and studies on the clinical efficacy of gur gum-based oral drug delivery systems of albendazole and albendazole-β.cyclodextrin for colon-targeting in human volunteers. *Drug Development Research.* 67:154-165.

Singh, D., Sanyal, P. K., Swarnkar, C. P., Khan, F. A., Bhagwan, P. S. K. (1999). Influence of type and pretreatment fasting on the disposition kinetic of albendazole in sheep. Veterinary Research Communications. 23:229-240.

Singounas EG, Leventis AS, Sakas DE, Hadley DM, Lampadarios DA, Karvounis PC. (1992). Successful treatment of intracerebral hydatid cyst with albendazole: case report and review of the literature. *Neurosurgery.* 31:571-574.

Skálová, L., Krizová, V., Cvilink, V., Szotáková, B., Storkánová, J., Velík, J., Lamka, J. (2007). Mouflon (Ovis musimon) dicroceliosis: Effects of parasitosis on the activities of biotransformation enzymes and albendazole metabolism in liver. Veterinary Parasitology. 146:254-262.

Skerrit, G. C. (1991). Coenurosis. In: Martin, W. B., Aitken, I. D. (eds) Diseases of Sheep. Second edition. Blackwell Scientific Publications. Oxford, UK.

Skerrit, G. C., Stallbaumer, M. F. (1984) Diagnosis and treatment of Coenurosis (gid) in Sheep. *Veterinary Record.* 11:399-403.

Smyth, J. P. (1994). Introduction to Animal Parasitology. Cambridge University Press, Cambridge, UK.

Solomon-Wisdom, .G.O, Matur, B.M and Ibe, .K.C (2014). Prevalence of Intestinal Helminth Infection Among Sheep and Goats Raised for Slaughtering in Gwagwalada Abattoir, Abuja-Nigeria. 02: 12-19. http://ejbios.co.in/jgpharma

Sotelo J (2004). Neurocysticercosis–is the elimination of parasites beneficial? The New England. Journal of Medicine. 350:280–282.

Sotelo, J., Jung, H. (1998). Pharmacokinetics optimisation of the treatment of neurocysticercosis. *Clinical Pharmacokinetics*. 34:503-515.

Souhaili-El Amri, H., Fargetton, X., Delatour, P., Batt, A. (1987). Sulphoxidation of albendazol by the FAD-containing and cytocrome P-450 dependent mono-oxygenases from pig liver microsomes. Xenobiotica. 17:1159-1168.

Souhaili-el Amri H, Fargetton X, Benoit E, Totis M, Batt AM. (1988a). Inducing effect of albendazole on rat liver drug-metabolizing enzymes and metabolite pharmacokinetics. Toxicology Applied Pharmacology. 92:141 -149

Souhaili-El Amri, H., Mothe, O., Totis, M., Masson, C., Batt, A., Delatour, P., Siest, G. (1988b). Albendazole sulfonation by rat liver cytochrome P-450c. *Journal of Pharmacology and Experimental Therapeutics.* 246:758-764.

Soulsby, E. J. L. (1982). Helminthes Artropods and Protozoa of Domesticated Animals. 7th Edition, Bailliere Tindall, London, UK.

Specht, E.J.K., (1982). Seasonal incidence of helminths in sheep and goat in South of Mozambique. *Veterinary Parasitology*. 11:317-328.

Sykes, A. (1978). The effect of subclinical parasitism in sheep. *Veterinary Record*. 102:32-34.

Tefera, T., & Mebrie, G. (2014). Prevalence and Predictors of Intestinal Parasites among Food Handlers in Yebu Town, Southwest Ethiopia. PLoS ONE, 9(10). e110621. http://doi.org/10.1371/journal.pone.0110621

Takayanagui, O. M., Bonato, P. S., Dreossi, S. A. C., Lanchote, V. L. (2002). Enantioselective distribution of albendazole metabolites in cerebrospinal fluid of patients with neurocysticercosis. British Journal of Clinical Pharmacology. 54:125-130.

Takemoto, Y., Negita, T., Ohnishi, K., Suzuki, M., Ito, A. (1995). A simple method for collecting eggs of taeniid cestodes from fresh, frozen or ethanol-fixed segments. *International Journal for Parasitology.* 25: 537-538.

Tavassoli, M., Malekifard, M., Soleimanzadeh, A., Tajik, H. (2011). Prevalence of Coenurus cerebralis in sheep in Northwest of Iran. Veterinary Research Forum. 2: 274 – 276

Taylor, M.A., Coop, R.L., Wall, R.L., (2007). Veterinary Parasitology. Third Edition Blackwell Publishing. Oxford, UK.

Taylor, S., Mallon, T., Blanchflower, J., Kennedy, D., Hewitts, S. (1993). Effects of dietary variations on plasma concentration of oral fluckicides in sheep. *Journal of Veterinary Pharmacology and Therapeutics*. 16:48-54.

Thompson, D., Ho, N., Sims, S., Geary, T. (1993). Mechanistic approaches to quantitate anthelmintic absorption by gastrointestinal nematodes. *Parasitology Today*. 9:31- 35.

Tirgari, M., Howard, B. R., Boargob, A. (1987). Clinical and radiographical diagnosis of coenurosis cerebralis in sheep and its surgical treatment. *Veterinary Record*. 120:173-178.

Todorov T, Vutova K, Mechkov G, Tonchev Z, Georgiev P, Lazarova I. (1992). Experience in the chemotherapy of severe, inoperable echinococcosis in man. *Infection*. 20:19-24.

Toofnian, F., Ivoghlib, B. (1976). Cerebral Coenurosis in a Wild Sheep (Ovis ammon). *Journal of South Africa Veterinary Association.* 12:550-551.

Torrado, S., López, M. L., Torrado, G., Bolás, F., Torrado, S., Cadórniga, R. (1997) A novel formulation of albendazole solution; oral bioavailability and efficacy evaluation. *International Journal of Pharmaceutics.* 156:181-187.

Trachsel, D., Deplazes, P., Mathis, A. (2007). Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology Research*. 134: 911-920.

Urquhart, G. M., Armour, J., Duncan, J. L., Jennings, F. W. (1996). Veterinary Parasitology. Second Edition. Longman, United Kingdom.

Uslu, U. D. U. R., & Guclu, (2007). Prevalence of Coenurus cerebralis in sheep in Turkey. Medycyna Weterynaryjna. 63: 678-680.

Varcasia, A., Tosciri, G., Coccone, G. S., Pipia, A. P., Garippa, G., Scala, A., Lightowlers, M. W. (2009). Preliminary field trial of a vaccine against coenurosis caused by Taenia multiceps. Veterinary Parasitology. 162: 285-289.

Varcasia, A., Jia, W.Z., Yan, H.B., Manunta, M.L., Pipia, A.P., Garippa, G., Scala, A.,Schuster, R.K. (2012). Molecular characterization of subcutaneous and muscular coenurosis of goats in United Arab Emirates. Veterinary Parasitology. 190,604–607.

Varcasia, A., Pipia, A.P., Arru, D., Pes, A.M., Tamponi, C., Dore, F., Garippa, G., Scala,. (2013). Morphological and molecular characterization of bovine coenurosisin Sardinia, Italy. *Parasitology Research.*112, 2079–2082

Varcasia, A., Lightowlers, M. W., Cattoli, G., Cancedda, G. M., Canu, S., Garippa, G., Scala, A. (2006). Genetic variation within Taenia multiceps in Sardinia, Western Mediterranean (Italy). *Parasitology Research*. 99:622-626.

Veerakumaru, L., Munuswamy, N. (2000). In vitro effect of some anthelmintics on lactate dehydrogenase activity of Cotylophoron cotylophoroum (Digenea: Paramphistomidae). *Veterinary Parasitology*. 91:129-140.

Vercruysse, J., Holdsworth, P.,Letonja, T., Barth, D., Conder, G.,Hamamoto, K., Okano, K. (2001). International harmonisation of Anthelmintic Efficacy Guidelines. Veteterinary Parasitology. 96, 171-193.

Verster, A., Tustin, R. C. (1982). Treatment of the larval stage of Taenia multiceps with praziquantel. Journal of South Africa Veterinary Association. 53:107-8.

Villaverde, C., Alvarez, A. I., Redondo, P. A., Voces, J., Del Estal, J. L., Prieto, J. G. (1995). Small intestinal sulphoxidation of albendazole. *Xenobiotica*. 25:433-441.

Vink, W. D., Lopes Pereira, C. M., Nota, A., Balogh, K. I. M. (1998). Prevalence of Coenuriasis in Goats in Tete Province, Mozambique. *Ciência e Técnica Veterinária*. 3:29-34.

Virkel, G., Imperiale, F., Lifschitz, A., Pis, A., Alvarez, A., Merino, G., Pietro, J., Lanusse, C. (2003). Effect of amphiphilic surfactant agents on the gastrointestinal absorption of albendazole in cattle. *Biopharmaceutics & Drug Disposition*. 24:95-103.

Virkel, G., Lifschitz, A., Pis, A., Sallovitz, J., Lanusse, C. (1997). Diet affects the ruminal biotrnasformation of netobimin and albendazole sulphoxide. Journal of Veterinary Pharmacology and Therapeutics. 20 (Suppl. 1):100-101.

Wang, Y., Nie, H., Gu, X., Wang, T., Huang, X., Chen, L., Yang, G. (2015). An ELISA using recombinant TmHSP70 for the diagnosis of Taenia multiceps infections in goats. Veterinary parasitology, 212: 469-472.

Wang, I. C., Ma, Y. X., Kuo, C. H., Fan, P. C. (1997). A comparative study on egg hatching methods and oncosphere viability determination for Taenia solium eggs. International Journal for Parasitology. 27: 1311-1314.

Welchman, B. D., Bekh-Ochir, G. (2006). Spinal Coenurosis causing posterior Paralysis in a goat in Mongolia. *Veterinary Record*. 158:238-239.

Wen, H., New, R. R. C., Muhmut, M., Wang, J. H., Zang, J. H., Shao, Y. M., Craig, P. S. (1996). Pharmacology and efficacy of liposome entrapped albendazole in experimental secondary echinococcosis and effect of co-administration with cimetididne. *Parasitology*. 13:111-121.

WHO (1996). Update/Le point. Guidlines for treatment of cystic and alveolar echinococcosis in humnas. *Bulletin of World Health Organization*. 74: 231-242.

Williams, J.C., DeRosa, A., Nakamura, Y., Loyacano, A.F. (1997). Comparative efficacy of ivermectin pour-on, albendazole, oxfendazole and fenbendazole against Ostertagia ostertagi inhibited larvae, other gastrointestinal nematodes and lungworm of cattle. *Veterinary Parasitology*. 73:73-82.

Willis, J. M., Herbert, I. V. (1987). A method for estimating the age of coenuri of Taenia multiceps recovered from the brains of sheep. *Veterinary Record*. 121:216-8.

Wu, X., Fu, Y., Yang, D., Zhang, R., Zheng, W., Nie, H., ... & Yang, G. (2012). Detailed transcriptome description of the neglected cestode *Taenia multiceps*. Plos one e45830.

Zhang, L., Hu, M., Jones, A., Allsopp, B.A., Beveridge, I., Schindler, A.R., Gasser, R.B. (2007) Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. *Molecular and Cellular Probes*. 21, 379-385.

ANNEXES

8. Annexes

8.1. Plasmatic concentration of albendazole metabolites

Annex 1 – Plasmatic concentrations of Albendazole metabolites

Table 1 – Plasmatic concentrations of ABZSO in adult female healthy goats after oral administration of ABZ 10 mg/kg

ABZSO								
time (h)	B1	B2	B3	B4	B5	B6	media	sd
-								
0,25			0,03				0,03	
0,5			0,03				0,03	
1	0,07	0,07	0,16	0,05	0,06		0,08	0,05
2	0,6	0,27	0,5	0,14	0,34	0,85	0,45	0,26
4	1,1	0,81	1,24	0,57	1,15	1,19	1,01	0,26
6	2,13	1,25	1,6	0,86	2,06	1,46	1,56	0,49
8	3,72	1,47	1,71	1,09	2,33	1,28	1,93	0,97
10	2,11	1,82	1,78	1,15	2,14	1,3	1,72	0,41
12	7,03	1,75	1,89	1,24	1,89	1,19	2,5	2,24
16	6,61	1,77	1,88	1,46	2,86	1,67	2,71	1,97
20	5,91	1,98	1,54	1,36	2,5	2,68	2,66	1,67
24	5,74	1,08	0,64	1,1	2,06	2,87	2,25	1,89
30	2,52	0,08	0,03	0,45	0,34	0,99	0,73	0,94
36	1,21		0,01	0,04	0,1	0,12	0,3	0,51
42	0,07						0,07	

Table 2 - Plasmatic concentrations of $ABZSO_2$ in adult female healthy goats after oral administration of ABZ 10 mg/kg

$ABZSO_2$								
time (h)	B1	B2	B3	B4	B5	B6	media	sd
-								
0,25								
0,5								
1								
2	0,02	0	0,03			0,05	0,03	0,02
4	0,12	0,07	0,12	0,04	0,06	0,16	0,09	0,05
6	0,21	0,18	0,22	0,12	0,22	0,31	0,21	0,06
8	1,01	0,31	0,35	0,24	0,23	0,37	0,42	0,3
10	0,54	0,53	0,5	0,32	0,39	0,5	0,46	0,09
12	2,54	0,66	0,58	0,44	0,45	0,63	0,88	0,82
16	3,58	1	1,15	0,75	1,05	0,98	1,42	1,07
20	4,29	1,68	1,17	0,95	1,36	2,36	1,97	1,24
24	5,91	1,85	0,2	1,14	1,73	3,25	2,35	2,01
30	4,55	0,87	0,01	1,09	1,96	2,32	1,8	1,58
36	4,77			0,44	0,99	0,95	1,79	2,01
42	0,95			0,02	0,02	0,2	0,3	0,44
48	0,02						0,02	

Table 3 - Plasmatic concentrations of ABZSO in young female healthy goats after oral administration of ABZ 10 mg/kg

ABZSO								
time (h)	F1	F2	F3	F4	F4	F6	media	sd
-	-	-	-		-			
0,5	0,28	0,56	1,38	0,33	0,11	1,28	0,66	0,54
1	1,73	1,96	4,43	0,82	0,89	1,71	1,92	1,32
2	1,98	2,57	4,85	1,5	1,87	4,38	2,86	1,41
4	1,52	2,55	3,33	2,08	2,12	5,35	2,83	1,38
6	1,68	2,55	3,93	2,27	2,9	5,32	3,11	1,32
8	1,23	2,18	3,96	3,63	2,9	5,49	3,23	1,48
10	0,92	2,06	3,8	3,16	3,04	3,69	2,78	1,1
12	0,57	1,69	3,07		3,06	2,97	2,27	1,12
16	0,22	0,25	1,72	2,94	4,31	2,23	1,94	1,59
20	0,02	0,16	0,02	0,97	2,22	2,4	0,96	1,1
24	-	-	-		1,41	0,64	0,41	0,62
30	-	-	-		0,15	-	0,03	0,07

Table 4 - Plasmatic concentrations of $ABZSO_2$ in young female healthy goats after oral administration of ABZ 10 mg/kg

$ABZSO_2$								
time (h)	F1	F2	F3	F4	F4	F6	media	sd
-				-		-		
0,5	0,05	0,06	0,15	0,06	0,04	0,07	0,07	0,04
1	0,17	0,21	0,55	0,16	0,19	0,19	0,24	0,15
2	0,4	0,54	1	0,41	0,49	0,61	0,57	0,23
4	0,58	0,8	1,09	0,7	0,78	1,04	0,83	0,2
6	0,8	1,11	1,41	1,03	1,06	1,45	1,14	0,25
8	0,84	1,32	1,9	1,86	1,39	2,13	1,57	0,47
10	0,89	1,52	2,23	1,94	1,76	1,68	1,67	0,46
12	0,86	1,7	2,78		2,06	1,67	1,81	0,7
16	0,73	1,68	2,44	2,73	3,51	1,58	2,11	0,98
20	0,14	0,06	0,18	2,61	3,6	2,77	1,56	1,61
24	0,01	0,02	0,01	0,11	3,48	2,15	0,96	1,49
30		0,01	0,02	-	2,06	0,03	0,43	0,92
36					0,11			
42					0,05			

Table 5 - Plasmatic concentrations of ABZSO in adult male healthy goats after oral administration of ABZ 10 mg/kg

ABZSO								
time (h)	V1	V2	V3	V4	V5	V6	media	sd
-								
0,25								
0,5						0	0	
1	0,1	0,12	0,04	0,21		0,13	0,12	0,06
2	0,26	0,15	0,23	0,64	0,16	0,7	0,35	0,25
4	1,1	0,74	0,74	2,15	1,04	1,93	1,28	0,61
6	1,81	0,92	2,57	2,36	1,57	2,41	1,94	0,63
8	2,05	1,48	2,35	3,36	1,82	3,36	2,4	0,8
10	2,17	1,23	1,59	3,94	2,72	2,6	2,38	0,96
12	2,12	1,33	2,15	3,83	1,88	3,36	2,44	0,95
16	2,01	1,52	2,77	3,76	3,53	3,75	2,89	0,96
20	2,01	1,87	2,99	3,84	2,55	5,99	3,21	1,54
24	1,85	3,29	3,38	2,9	2	2,86	2,71	0,65
30	0,45	0,23	1,85	0,77	2,26	0,87	1,07	0,8
36	0,03		0,62	0,03	0,91		0,4	0,44
40			0,09	0,02	0,87		0,32	0,47
48					0,24		0,24	

Table 6 - Plasmatic concentrations of $ABZSO_2$ in adult male healthy goats after oral administration of ABZ 10 mg/kg

$ABZSO_2$								
time (h)	V1	V2	V3	V4	V5	V6	media	sd
-								
0,25								
0,5								
1								
2	0,02	0,03	0,03	0,02	0,07	0,04	0,03	0,02
4	0,11	0,03	0,06	0,17	0,23	0,18	0,13	0,07
6	0,26	0,15	0,33	0,27	0,33	0,32	0,28	0,07
8	0,45	0,36	0,4	0,61	0,46	0,7	0,49	0,13
10	0,64	0,42	0,37	0,91	0,72	0,71	0,63	0,2
12	0,86	0,55	0,59	1,08	0,6	1,23	0,82	0,29
16	1,2	0,94	0,93	1,57	1,35	2,02	1,34	0,42
20	1,62	1,49	1,29	2,41	1,15	4,84	2,13	1,39
24	2,13	3,59	2,09	2,92	1,31	3,68	2,62	0,94
30	1,39	1,13	2,27	2,09	1,82	3,2	1,98	0,73
36	0,12	0,03	1,9	0,12	1,57	0,03	0,63	0,86
42		0	0,62	0,02	2,62	0,02	0,66	1,13
48	0,02		0,03		1,59		0,55	0,9
54					0,28		0,28	

Table 7 - Plasmatic concentrations of ABZSO in young male healthy goats after oral administration of ABZ 10 mg/kg

ABZSO								
time (h)	M1	M2	M3	M4	M5	M6	media	sd
-			-				-	
0,5	-	0,75	0,36	0,17	0,4	1,4	0,51	0,5
1	0,12	1,32	1,22	0,53	0,74	3,54	1,25	1,21
2	0,36	2,37	1,98	0,69	0,92	7,17	2,25	2,53
4	0,28	2,68	1,95	0,93	0,69	6,08	2,1	2,14
6	0,45	2,82	2,26	1,22	1,2	4,22	2,03	1,37
8	0,74	2,47	3,05	1,16	1,23	5,1	2,29	1,63
10	1,22	2,6	2,87	1,91	1,07	4,09	2,29	1,14
12	0,97	2,25	2,08	2,43	1	3,63	2,06	0,99
16	1,28	1,61		1,99	0,63	2,86	1,67	0,83
20	0,9	0,23		2,06	0,11	0,58	0,78	0,78
24	0,58			0,92			0,75	0,24
30	0,25				0,05		0,15	0,14
36	0,13				0,03		0,08	0,07
42	0,04						0,04	

Table 8 - Plasmatic concentrations of $ABZSO_2$ in young male healthy goats after oral administration of ABZ 10 mg/kg

$ABZSO_2$								
time (h)	M1	M2	M3	M4	M5	M6	media	sd
-	-		-		-		-	-
0,5	-	0,04	0,08	0,09	0,08	0,05	0,06	0,03
1	0,05	0,15	0,26	0,21	0,2	0,47	0,22	0,14
2	0,23	0,47	0,6	0,37	0,43	1,61	0,62	0,5
4	0,27	0,73	0,77	0,64	0,56	1,86	0,81	0,55
6	0,36	1,08	0,88	0,86	0,82	1,8	0,96	0,47
8	0,59	1,24	1,34	0,76	1,04	2,66	1,27	0,74
10	1,07	1,55	1,5	1,23	0,98	2,58	1,49	0,58
12	0,92	1,82	1,31	1,71	1,42	2,9	1,68	0,67
16	1,51	2,02		2,58	1,49	3,14	2,15	0,71
20	1,53	2,46		2,81	0,82	2,47	2,02	0,82
24	1,55			2,78	0,04	0,1	1,12	1,31
30	1,39			0,07			0,73	0,94
36	1,34						1,34	
42	0,69						0,69	
48	0,6						0,6	
54	0,27						0,27	

Table 9 - Plasmatic concentrations of ABZSO in adult male goats infected with *T. multiceps* goats after oral administration of ABZ 10 mg/kg

ABZSO									
time (h)	A	B	C	D	E	F	G	media	sd
-	-	-	-	-	-	-	-	-	-
1	0,07	0,02	-	0,05			-	0,03	0,03
2	0,21	0,21	0,22		0,22	0,45	-	0,22	0,14
3	0,37	0,33	0,51	0,48				0,42	0,09
4	0,36	0,45	0,79	0,76	0,59	1,24	0,12	0,62	0,36
6	0,6	0,56	0,98	1,14		1,21	0,27	0,79	0,37
8	0,69	0,82	1,17	1,41	0,01	1,57	0,35	0,86	0,56
10	1,08	1,04	1,6		1,06	1,32	0,74	1,14	0,29
12	1,02	1,27	1,53	1,59	1,31	0,96	1,17	1,26	0,24
16		1,21	1,98		1,73	1,67		1,65	0,32
20	1,86	1,19	1,68	1,23	1,65	1,07	1,63	1,47	0,3
24	0,85	0,8	1,35	1,07	1,66	0,75	0,99	1,07	0,33
30	0,63	0,76	1,1	0,61	1,28	0,4	1,15	0,85	0,33
36	0,33	0,1	0,44		1,44	0,04	0,36	0,45	0,51
42	0,07	-	0,06	0,02	0,27	-	0,63	0,15	0,23
48	-	-	-	-	0,05	-	0,36	0,06	0,13

Table 10 - Plasmatic concentrations of $ABZSO_2$ in adult male goats infected with *T. multiceps* after oral administration of ABZ 10 mg/kg

$ABZSO_2$									
time (h)	A	B	C	D	E	F	G	media	sd
-	-	-	-	-	-		-	-	-
1	-	0,01	0,01		-		-	0	0
2	0,03	0,03	0,03		0,05	0,04	0,01	0,03	0,02
3	0,04	0,05	0,04	0,05				0,04	0
4	0,04	0,06	0,08	0,08	0,18	0,12	0,02	0,08	0,05
6	0,07	0,11	0,15	0,15		0,15	0,05	0,11	0,04
8	0,1	0,18	0,22	0,24	0,01	0,25	0,07	0,15	0,09
10	0,19	0,25	0,36		0,34	0,29	0,14	0,26	0,08
12	0,21	0,42	0,39	0,41	0,44	0,25	0,24	0,34	0,1
16		0,51	0,69		0,72	0,66		0,64	0,09
20	0,71	0,73	0,78	0,73	0,77	0,59	0,56	0,7	0,09
24	0,55	0,87	1,05	1,14	1,13	0,73	0,54	0,86	0,26
30	0,82	1,5	1,4	1,35	1,33	0,82	1,03	1,18	0,28
36	0,98	0,75	1,32		2,55	0,28	0,6	1,08	0,8
42	0,51	0,06	0,85	0,16	1,08	0,06	1,44	0,59	0,54
48	0,1	0,01	0,19	0,03	0,48	0,03	1,4	0,32	0,5
54	-	0,01	0,02	-	0,03		-	0,01	0,01

Table 11 - Plasmatic concentrations of ABZSO in adult male goats infected with *T. multiceps* after oral administration of ABZ 20 mg/kg

ABZSO									
time (h)	A	B	C	D	E	F	G	media	sd
-	-	-	-	-	-	-	-	-	-
1	-	0,11	-	0,05	-	0,01	0,08	0,04	0,05
2	0,12	0,3	0,12	0,2	0,11	0,16	0,26	0,18	0,08
3	0,3	0,48	0,26	0,45	0,41	0,49	0,5	0,41	0,1
4	0,54	0,91	0,4	0,69	0,51	0,5	0,72	0,61	0,17
6	1,01	0,97	0,85	1,18	1,51	0,81	1,25	1,08	0,25
8	1,65	-	1,23	1,42	1,73	0,95	1,94	1,28	0,65
10	1,87	2,5	1,43	1,56	4,81	1,12	2,47	2,25	1,24
12	2,21	3,16	1,85	1,88	1,89	1,78	3,19	2,28	0,63
16		3,64	2,71	1,93	2,08	2,26	4,06	2,78	0,88
20		2,95	2,61	2,67	4,17		4,35	3,35	0,84
24	2,78	3,52	2,96	2,4	0,83	2,99		2,58	0,93
30	1,8	4,02	2,1	2,38	1,48	2,94	3,33	2,58	0,9
36	1,31	2,24	2,14	2,41	0,79	2,53		1,9	0,7
42	0,34	1,67		1,46	1,54		0,63	1,13	0,6
48	-	1,02	0,96	0,92	0,26	1,15	1,12	0,78	0,45
54	-	0,27	0,25	0,55	-	0,2	0,4	0,24	0,2

Table 12 - Plasmatic concentrations of $ABZSO_2$ in adult male goats infected with *T. multiceps* after oral administration of ABZ 20 mg/kg

$ABZSO_2$									
time (h)	A	B	C	D	E	F	G	media	sd
-	-	-	-	-	-	-	-	-	-
1	0,01	-	0,02	-	-	0,01	-	0,01	0,01
2	0,04	0,03	0,01	0,02	0,01	0,02	0,02	0,02	0,01
3	0,06	0,04	0,03	0,03	0,03	0,05	0,03	0,04	0,01
4	0,09	0,08	0,05	0,06	0,05	0,05	0,06	0,06	0,02
6	0,17	0,11	0,09	0,13	0,19	0,11	0,12	0,13	0,04
8	0,34	-	0,16	0,26	0,21	0,17	0,22	0,19	0,11
10	0,46	0,35	0,21	0,26	0,82	0,22	0,31	0,38	0,21
12	0,67	0,53	0,3	0,36	0,39	0,35	0,49	0,44	0,13
16		0,85	0,59	0,45	0,6	0,56	0,84	0,65	0,16
20		0,9	0,76	0,79	1,51		1,14	1,02	0,31
24	2,37	1,53	1,28	1,12	0,47	1,41		1,36	0,62
30	2,2	2,26	1,19	1,34	1,15	1,64	1,71	1,64	0,45
36	2,81	1,87	1,82	1,72	1,16	1,99		1,9	0,53
42	2,04	2,09		1,66	1,86		1,41	1,81	0,28
48	0,12	2,17	1,97	1,52	1,15	1,93	1,95	1,54	0,72
54	0,01	1,33	1,41	1,76	0,28	1,35	1,69	1,12	0,69

Table 13 - Plasmatic concentrations of ABZSO in adult male goats infected with *T. multiceps* after oral administration of ABZ 10 mg/kg for 3 consecutive days

ABZSO									
time (h)	**A**	**B**	**C**	**D**	**E**	**G**	**H**	**media**	**sd**
-	-	-	-	-	-	-	-	-	-
1	0,12	0	-	0,08	0,08	0,06	1,09	0,2	0,39
2	0,35	0,09	0,92	0,13	0,17	0,31	1,61	0,51	0,56
4	0,83	0,31	2,04	0,11	1,05	0,87	1,42	0,95	0,65
6	1,07	0,38	2,92	0,04	0,39	1,49	1,87	1,17	1,01
8	1,2	0,37	2,16	0,7	0,69	1,83	1	1,14	0,65
10	0,78	1,1	1,49	1,16	0,75	1,76	1,59	1,23	0,4
12	1,51	1,27	1,48	1,45	1,07	1,73	1,28	1,4	0,21
16	1,3	1,5	1,26	1,68	1,17	2,07	0,6	1,37	0,46
20	1,19	1,61	1,34	1,86	1,14	1,44	0,14	1,25	0,55
24	0,81	1,45	1,3	1,53	0,95	1,21	0,12	1,05	0,48
25	0,89	0,89	1	1,43	0,93	1,64	0,19	1	0,46
26	0,99	1,31	0,8	1,26	0,91	1,37	0,49	1,02	0,32
28	1,1	1,37	1,21	1,39	0,8	1,8	1,43	1,3	0,31
30	1,14	1,63	1,63	1,59	0,91	2,4	1,51	1,54	0,47
32	1,13	1,84	1,9	1,69	0,83	2,46	2,4	1,75	0,6
34	1	1,71	1,77	1,44	0,06	2,24	1,38	1,37	0,7
36	**1,06**	1,36	1,7	1,4	0,3	2,05	1,75	1,37	0,57
40	**0,44**	1,24	1,61	1,59	0,35	1,39	0,99	1,09	0,52
44	**0,2**	1,14	1,25	1,61	0,3	0,43	0,32	0,75	0,57
48	**0,17**	0,56	0,67	1,04	0,19	0,06	0,07	0,39	0,37
49	**0,16**	0,44	0,95	1,13	0,18	0,05	0,39	0,47	0,41
50	**0,31**	0,76	0,55	1,06	0,26	0,19	0,99	0,59	0,36
52	**0,77**	1,31	1,03	1,68	0,58	0,62	1,62	1,09	0,46
54	**1**	1,7	1,33	2,13	0,93	1,21	2,33	1,52	0,55
56	**1,21**	1,91	1,46	2,25	1,15	1,53	2,04	1,65	0,42
58	**1,03**	1,76	1,18	2,47	0,89	1,51	1,26	1,44	0,54
60	**1,1**	1,88	1,63	2,41	0,91	1,44	1,86	1,6	0,51
64	**1,3**	0,96	1,54	1,92	0,89	1,02	0,7	1,19	0,42
68	**0,51**	0,39	0,93	0,95	0,63	0,61	0,07	0,58	0,31
72	**0,12**	0,07	0,46	0,31	0,55	0,09	-	0,23	0,21
78	-	-	0,05	0,08	0,27	0,07	-	0,07	0,1
84	-	-	0,01	0,07	-	-	-	0,01	0,03
90	-	-	0,03	0,06	-	-	-	0,01	0,02

Table 14 – Plasmatic concentrations of $ABZSO_2$ in adult male goats infected with *T. multiceps* after oral administration of ABZ 10 mg/kg for 3 consecutive days

$ABZSO_2$									
time (h)	A	B	C	D	E	G	H	media	sd
-	-	-	-	-	-	-	-	-	-
1	0,02	0,02	0,02	-	-	-	0,04	0,01	0,02
2	0,05	0,04	0,1	0,01	0,12	0,03	0,15	0,07	0,05
4	0,14	0,11	0,15	0,1	0,23	0,13	0,29	0,16	0,07
6	0,25	0,15	0,22	0,13	0,24	0,31	0,57	0,27	0,15
8	0,37	0,18	0,41	0,27	0,38	0,53	0,48	0,38	0,12
10	0,31	0,35	0,44	0,4	0,4	0,67	0,95	0,5	0,23
12	0,7	0,48	0,66	1,04	0,62	0,89	1,16	0,79	0,25
16	0,98	0,72	0,9	1	0,99	1,76	1,18	1,07	0,33
20	1,33	1,15	1,3	1,59	1,52	1,78	0,61	1,33	0,38
24	1,42	1,42	1,94	2,2	2,2	2,25	0,05	1,64	0,79
25	1,46	0,96	1,73	1,98	1,94	3,09	0,22	1,63	0,9
26	1,49	1,57	1,38	1,77	1,88	2,31	0,4	1,54	0,59
28	1,55	1,61	1,83	1,97	1,67	2,25	0,99	1,7	0,39
30	1,65	1,92	2,05	2,27	2,16	2,98	1,18	2,03	0,56
32	1,95	2,28	2,26	2,52	2,53	3,16	2,35	2,44	0,37
34	1,88	2,6	2,17	2,36	2,31	3,73	1,74	2,4	0,65
36	1,98	2,26	2,43	2,72	1,53	3,89	2,59	2,49	0,74
40	1,74	2,51	2,8	3,27	1,71	3,58	2,68	2,61	0,71
44	1,02	2,86	2,89	3,91	1,49	2,14	1,41	2,24	1,03
48	1,01	1,92	2,32	3,15	1,11	0,63	0,54	1,53	0,97
49	0,74	2,22	2,22	3,35	0,86	0,39	0,82	1,51	1,09
50	0,87	1,55	2,19	3,11	1,07	0,74	1,05	1,51	0,86
52	1,22	2,26	2,51	3,63	1,59	1,14	1,52	1,98	0,89
54	1,49	2,54	2,51	3,82	2,18	1,87	2,41	2,4	0,73
56	1,84	2,66	2,56	3,75	2,82	2,56	2,51	2,67	0,57
58	1,66	3,15	2,15	4,24	2,37	2,75	2,15	2,64	0,85
60	1,95	3	3,1	4,53	2,98	3,14	3,59	3,19	0,77
64	3,34	2,4	3,68	4,53	3,44	2,95	2,49	3,26	0,74
68	2,49	1,59	3,11	3,49	2,86	2,34	0,57	2,35	0,99
72	0,83	0,79	2,46	1,67	3,08	0,83	-	1,38	1,08
78	0,11	0,03	0,89	0,01	-	0,68	0,22	0,28	0,36
84	-	-	0,01	0,03	-	-	-	0,01	0,01
90	0,01	-	0,03	0,02	-	-	-	0,01	0,01

Table 15 – Plasmatic concentrations of ABZSO, during 12 hours, in adult male goats infected with *T. multiceps* goats after oral administration of ABZ 10 mg/kg

ABZSO							
time (h)	43	14	33	18	19	media	sd
1	0,07	0,1	0,11	0,15	0,07	0,1	0,03
2	0,35	0,39	0,59	0,59	0,5	0,48	0,11
6	0,52	0,56	0,86	0,81	1,03	0,76	0,21
8	1,19	0,98	1,47	1,04	1,28	1,19	0,19
10	1,58	1,33	1,56	1,34	1,61	1,48	0,14
12	1,96	3,6	1,67	1,26	1,53	2	0,93

Table 16 - Plasmatic concentrations of $ABZSO_2$, during 12 hours, in adult male goats infected with *T. multiceps* goats after oral administration of ABZ 10 mg/kg

$ABZSO_2$							
time (h)	43	14	33	18	19	media	sd
1	0,02	0,02	0,02	0,03	0,03	0,02	0,01
2	0,07	0,05	0,08	0,09	0,07	0,07	0,02
6	0,11	0,08	0,13	0,2	0,16	0,14	0,04
8	0,25	0,15	0,24	0,22	0,23	0,22	0,04
10	0,44	0,26	0,33	0,38	0,36	0,36	0,07
12	0,75	0,94	0,46	0,5	0,45	0,62	0,22

8.2. Articles included in experimental part of the thesis

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Research Article

Prevalence and Morphological Characteristics of *Taenia multiceps* Cysts (Coenurus Cerebralis) from Abattoir-Slaughtered and Experimentally Infected Goats

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Abstract One hundred and forty nine indigenous goats slaughtered at the Tete municipal abattoir, Mozambique, and fifty seven goats experimentally infected with *Taenia multiceps* eggs were inspected to determine the prevalence, predilection sites and morphological characteristic of cysts and scoleces of *T. multiceps*. The experimentally infected goats of mixed sexes were divided into Group one (28 animals slaughtered at 10 weeks post-infection) and Group two (29 animals slaughtered at 22 weeks post-infection). Each animal was experimentally infected with 3000 eggs of *T. multiceps* recuperated from experimentally infected dogs. The prevalence of infection was 14.8% in the naturally infected goats, 78.6% for Group 1 and 82.8% for Group 2 of the experimentally infected goats. The muscle and subcutaneous tissues were the most common anatomic location of cysts in both the naturally and experimentally infected (Group 1 and 2) goats with a prevalence of 9.1%, 82% and 75% respectively and this was followed by the brain in the naturally infected (8.5%), and Group 2 (11.4%) and the heart and mesentery (4.5%) in Group 2. In the abattoir-slaughtered goats, animals with one cyst were more frequent in both muscular and subcutaneous tissues (73.3%) and brain (75%) and fewer animals were found to harbor two or more cysts.

Keywords *Taenia multiceps*; coenurus cerebralis; cysts; meat inspection; goats; Mozambique; predilections sites

1 Introduction

Taenia multiceps (Leske, 1780) (syn. ***Multiceps multiceps***) is a worldwide parasite which inhabits the small intestine of dogs, foxes, coyotes and jackals [22]. The larval stage, known as coenurus cerebralis, causes a central nervous system disease in sheep commonly known as coenurosis, gid or sturdy and may also affect other animal species such as domestic and wild ruminants including humans [5, 14, 23, 26, 29, 30]. Apart from the cerebral form, which is usually fatal, intramuscular and subcutaneous connective tissues, liver, lung forms have been reported to occur [26].

Animal cerebral coenurosis is usually diagnosed based on a clinical examination protocol [16, 24, 25] and seldom includes imaging methods like radiology [28], ultrasonography [8] and computed tomography [12] which are mainly used in experimental situations. Immunodiagnosis tests such as skin test for immediate hypersensitivity, indirect haemaglutination antibody (IHA) test, immuno-electrophoresis (IEP), gel double diffusion (DD), immunoblot and enzyme linked immune-assay (ELISA) tests have been used experimentally [6, 7, 21, 25]. Despite the availability of these tests which have their own practical challenges, post mortem findings of a thin walled cyst filled with transparent fluid and with numerous scoleces in the wall remain the definitive diagnosis [15, 26].

The prevalence of *T. multiceps* larva in Gaza and Tete Provinces of Mozambique has been reported to range from 7.3% to 13.8% based on abattoir findings [3, 32]. To the best of our knowledge, no other data exist on the occurrence of coenurosis in Mozambique and no references have been made to the site of predilection and the morphological characteristics of the larva and scoleces in goats. For this reason, the present study was conducted to determine the prevalence and predilection sites of *T. multiceps* cysts in goats slaughtered at a local abattoir and those experimentally infected and the morphological characteristics of the cysts and scoleces. The results will add new information on the predilection sites of *T. multiceps* in goats and assist meat inspectors in locating the cysts at abattoirs during meat inspection.

Groups	*N*	Infective dose/animal	Week of slaughter post-infection
1. Experimentally infected	28	3000	10
2. Experimentally infected	29	3000	22
3. Abattoir-slaughtered	149*	Not known	Not known

*Refer to organs and not number of goats.

Table 1: Experimental design of the study.

2 Material and methods

2.1 Meat inspection at abattoir

Various organs and muscle tissues from a total of 169 abattoir-slaughtered goats of local breeds of mixed sexes from a municipal abattoir in Tete Province, central west region of Mozambique, were inspected for the presence of *T. multiceps* cysts [22]. The number of organs and muscle tissues accessed for inspection varied as it depended on willingness of owners to access them for thorough inspection.

2.2 Experimental animals

2.2.1 Goats

The experimental study was conducted at the Veterinary Faculty, Eduardo Mondlane University (EMU), Mozambique. Fifty seven male goats of local breed, *Landin*, aged between 8 and 18 months old were purchased from *T. multiceps*-free farms. In order to assess the morphological characteristics of *T. multiceps* cysts at different weeks post-infection, the animals were randomly divided into two groups (Group one, 28 animals and Group two, 29 animals) (Table 1). Animals were housed at the Veterinary Faculty, EMU campus, under good clinical practice with free access to food (hay and grain ration) and water. The adaptation period, from purchase to infection, was four months. The study was approved by the Scientific Committee from the Veterinary Faculty, EMU.

2.2.2 Dogs

Six puppies of mixed breed (3 for each experiment) from the same breed and litter were selected for experimental infection with *T. multiceps* scoleces to generate eggs for experimental infection of goats. The puppies were weaned at 2 months and kept in separate kennels at the EMU veterinary hospital. They were fed commercial dog food, supplied with clean water *ad libitum* and treated following prophylactics measures established at the EMU veterinary hospital as follows: multiple vaccination against the common viral diseases including rabies and deworming with a combination of pyrantel, oxantel and praziquntel (Canex-4®) one month before the experimental infection. The health of the dogs was routinely monitored throughout the period of the study.

2.3 Experimental infection in dogs

Taenia multiceps cysts collected from naturally-infected goats from Tete municipal abattoir were immediately processed after collection by removing tissue surrounding the cysts. This was followed by washing the cysts in phosphate buffer saline (PBS), pH 7.3, preserving them in a sterile recipient and maintaining them at 4 °C until time of infection.

The dogs were each orally infected with 100 scoleces at the age of four months. Patency was evaluated by collecting faecal samples from infected dogs once a week to detect taeniidae eggs using the McMaster flotation technique [15]. After the parasite had reached patency, shown by presence of taenid eggs in faeces, the dogs were euthanized with 20% pentobarbital (Eutha-Naze) at a dose of 200 mg/kg. *Taenia multiceps* adult parasites were removed from the small intestine and gravid proglotids were separated to recover the eggs.

Standard operating procedures regarding the safety of researchers were strictly followed at all stages of the experimental infection. The dog kennels were kept isolated and access was restricted to the researchers only. All faeces from the infected dogs were incinerated as from day one post-infection.

2.4 Experimental infection in goats

Gravid proglottids of *T. multiceps* were crushed in 2.5% potassium dichromate to release the eggs and the eggs were preserved in a refrigerator at 4 °C before being subjected to the hatching process. In vitro hatching was performed using sodium hypochlorite [17,27,33]. Briefly, eggs were centrifuged at 1000 rpm for 5 minutes and 0.5 mL of sodium hypochlorite (0.5% of sodium hypochlorite in normal saline) and 10 mL of formol saline were added. The solution was centrifuged at 1000 rpm for 5 minutes. The sediment, containing the hatching eggs, was washed three times in normal saline and counted [17,27,33]. Each goat was orally infected with 3000 viable eggs via a cellulose and amide bolus.

Group one animals were slaughtered at 10 weeks post-infection and Group two at 22 weeks post-infection. The procedure for localization of cysts from the various organs and tissues was done following the anatomic classification according to following authors [19,20]. Cysts were removed from the tissue and then washed with phosphate saline buffer (PBS) pH 7.3 and placed in a Petri dish. The number of scoleces from each cyst was determined and the length and width of the scoleces were measured with a ruler under a stereomicroscope.

Location	Prevalence of cysts (%)		
	Abattoir-slaughtered *N* (% infected)	Experiment 1 *N* (% infected)	Experiment 2 *N* (% infected)
Muscle and subcutaneous tissue*	149 (9.1)	28 (82.0)	29 (75.0)
Brain	142 (8.5)	28 (5.0)	29 (11.4)
Mesentery	115 (1.7)	28 (0.0)	29 (4.5)
Lungs	149 (0)	28 (1.0)	29 (2.3)
Diaphragm	115 (0)	28 (5.0)	29 (2.3)
Heart	149 (0)	28 (7.0)	29 (4.5)
Overall prevalence	169 (14.8)	22 (78.6)	24 (82.8)

N = sample size; Experiment 1 = 10 weeks post-infection; experiment 2 = 22 weeks post-infection; *Muscle and subcutaneous tissue include muscles from the neck region, thoracic region, abdominal wall, lumbar and pelvic region.

Table 2: Prevalence of *Taenia multiceps* cysts (coenurus cerebralis) at various locations in abattoir-slaughtered and experimentally infected goats.

2.5 Data analysis

Descriptive statistics which included the determination of prevalence of *T. multiceps* cysts in abattoir-slaughtered and the experimentally infected goats were calculated. Data on the location of cysts from the abattoir-slaughtered and the experimentally infected goats were compared using a chi-square test with the level of significance set at $P < .05$. The computer software SPSS 13.0 SPSS Inc., Chigago, Il, was used for data analysis.

3 Results

The overall prevalence of *T. multiceps* cysts in abattoir-slaughtered goats determined through meat inspection was 14.8% (22/149) and of experimentally infected goats was 78.6% (22/28) and 82.8% (24/29) for group one and two respectively (Table 2). The muscle and subcutaneous tissues were the most common anatomic location of cysts in both the naturally and experimentally (Group one and two) infected goats with a prevalence of 9.1%, 82% and 75% respectively and this was followed by the brain in the abattoir-slaughtered group (8.5%), and Group two (11.4%) and the heart (7.0%) and diaphragm (5.0%) in Group one animals. A low number of cysts was observed in the brain (5% for Group one and 11% for Group two). Overall, *T. multiceps* cysts were distributed to a minimum of five anatomical sites in the experimentally infected goats whilst they were only observed at three sites in the naturally infected goats. It is worth mentioning that muscle and subcutaneous tissue included muscles from the neck, thoracic, lumbar and pelvic region and abdominal wall. A significant difference ($P < .05$) was observed between the number of cysts observed in the brain of abattoir-slaughtered goats and experimentally infected animals with the abattoir-slaughtered animals having more cysts in the brain. There was no significant difference ($P > .05$) in the number of cysts observed in the muscles and subcutaneous tissue between the two groups.

The morphological characteristics of the *T. multiceps* cysts from naturally and experimentally infected goats were similar. Cysts were composed of a hyaline membrane with many scoleces on the inner surface and filled with a translucid fluid of varying volume. In both cases, cysts which were located outside of the brain were surrounded by a thick host membrane.

In the naturally infected group, animals with one cyst were more frequent in both muscular and subcutaneous tissues (73.3%) and brain (75%) and few animals were found to harbor two or more cysts. In the experimentally infected groups, animals with two cysts (11 animals) were the most common and only three goats had 10, 12 and 16 cysts each, respectively.

Size of larvae and number of scoleces per cyst increased with the age as observed at 10 and 22 weeks post-infection (Table 3). Young cysts of 10 weeks of age had fewer scoleces (51.7 ± 27.7) than the older ones of 22 weeks (92.2 ± 48.7).

4 Discussion

In Africa, *T. multiceps* cysts in the central nervous system (CNS) have been reported in domestic ruminants in Kenya, Ethiopia, Democratic Republic of Congo, Senegal, Sudan, Chad, Angola, Southern Africa [30] and Mozambique [32]. However, none of these studies contain information regarding the predilection sites of larval stages. The prevalence of *T. multiceps* cysts in naturally infected goats in this study (14.8%) was higher than the prevalence reported in a retrospective study in sheep in Ethiopia (2.34–4.54%) [2] and in goats in Gaza (8%) and Tete (7.3%) provinces of Mozambique [3] and comparable to the prevalence reported of (13.8%) in a study conducted in Tete abattoir, Mozambique [32]. Prevalence studies of *T. multiceps* infection in small ruminants in Southern African countries are very limited [32] and this study gives an indication that the parasite is wide spread, especially in the

Time of slaughter post-infection	N	Mean length ± sd (min-max)	Mean width ± sd (min-max)
	Mean length (range) and width (range) of larva at 10 and 12 weeks post-infection		
10 weeks	66	2.1 ± 1.06 (0.1–5.8)	1.4 ± 0.75 (0.1–5.8)
22 weeks	46	3.38 ± 1.4 (1.0–6.8)	2.44 ± 1.12 (1.0–6.8)
	Mean number (range) of scoleces per goat at 10 and 12 weeks post-infection		
	N	Scoleces	
10 weeks	83	51.73 ± 27.7 (6–181)	
22 weeks	36	92.22 ± 48.71 (18–206)	

Table 3: Size of *Taenia multiceps* cysts (coenurus cerebralis) and number of scoleces per cyst in experimentally infected goats at 10 and 22 weeks post-infection.

resource-poor rural communities and the zoonotic impact of the parasite is yet to be assessed.

The common predilection sites of *T. multiceps* cysts in sheep is the CNS, whilst in goats, apart from the CNS, the muscle and subcutaneous form have been reported [23,26]. Subcutaneous cysts found in an infected goat were classified as ***Multiceps gaigeri*** [13] and in subsequent studies similar cysts were found in several organs including muscles and subcutaneous tissue and were re-classified as *T. multiceps* [10,18,23].

Results from our study shows that a larger proportion of *T. multiceps* cysts were found outside the CNS and mostly in muscle and subcutaneous tissues in the experimentally infected goats than the naturally infected. This difference is likely to be due to the fact that the cysts in the muscles of the experimentally infected animals were located in the medium and deep layers of muscles which increase the possibility of becoming unnoticed at abattoir inspection. The low sensitivity of abattoir routine meat inspection for *T. multiceps* cysts is an important factor that may contribute in perpetuating the cycle of the parasite.

Previous experimental infections with *T. multiceps* larva were done in sheep using a high dose of 5500 eggs per sheep [4] and 6500 eggs [11]. In the two studies the infection rate was 100% in contrast to lower infection rates recorded in our study. This difference might have been associated to the combination of a relatively low infection dose, egg viability or regurgitation of the infected bolus.

Previous studies show that clinical coenurosis in sheep is common in young animals [1,22]. Age-related resistance to infection with *T. multiceps* in sheep has been suggested [31], however, the mechanism by which this may come about is not apparent and cross immunity within taeniidae cestodes has been discussed by some authors [9]. The increase with age in cyst size and the number of scoleces found in this study are in accordance with a previous study in sheep [34].

Our findings have shown the prevalence of *T. multiceps* cysts in goats is high in Mozambique and this might be the same situation in most countries in Southern Africa where goats are extensively reared. In addition, an important finding from the experimental infection study was that the predilection sites, apart from the brain include skeletal muscles and subcutaneous tissue, lungs, diaphragm, heart and mesentery and that the current routine abattoir inspection targeting only the brain is not a reliable method in detecting all cases *T. multiceps* infection in goats. Development and validation of a sensitive and specific diagnostic method for *T. multiceps* infection in goats should be investigated in order to improve the surveillance and control of the parasite.

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References

[1] M. N. Abo-Shehada, E. Jebreen, B. Arab, R. Mukbel, and P. Torgerson, *Prevalence of Taenia multiceps in sheep in northern Jordan*, Prev Vet Medicine, 55 (2002), 201–202.

[2] M. Achenef, T. Markos, G. Feseha, A. Hibret, and S. Tembely, *Coenurus cerebralis infection in Ethiopian highland sheep: incidence and observations on pathogenesis and clinical signs*, Trop Anim Health Prod, 31 (1999), 15–24.

[3] A. Atanásio, *Helminths, protozoa, heartwater and the effect of gastrointestinal nematodes on productivity of goats of family sector in Mozambique*, PhD thesis, Medical University of Southern Africa, Medunsa, South Africa, 2000.

[4] G. Biyikoğlu and A. Doğanay, *Effects of praziquantel and albendazole on Coenurus cerebralis in experimentally infected lambs*, Turk J Vet Anim Sci, 22 (1998), 43–48.

[5] R. Bohrmann, *Coenurus in the muscles of a gemsbok (Oryx gazelle)*, Vet Parasitol, 36 (1990), 353–356.

[6] I. S. Daoud and I. V. Herbert, *Isolation of two lipoprotein antigens from the metacestodes of Taenia hydatigena (Pallas, 1766) and Taenia multiceps (Leske, 1780) and their evaluation in sero-diagnosis*, Vet Parasitol, 11 (1982), 155–164.

[7] A. Doganay, H. Oge, and G. Biyikoglu, *Serodiagnosis of coenurosis by ELISA in experimental infected lambs*, Turkiye Parazitol Derg, 23 (1999), 185–189.

[8] M. L. Doherty, H. McAllister, and A. Healy, *Ultrasound as an aid to Coenurus cerebralis cyst localisation in a lamb*, Vet Rec, 124 (1989), 591.

[9] G. T. Edwards and I. V. Herbert, *Observations on the course of Taenia multiceps infections in sheep: clinical signs and post-mortem findings*, Br Vet J, 138 (1982), 489–500.

[10] K. A. el Sinnary, M. H. Tageldin, and H. S. al Sumry, *Outbreak of coenurosis (Taenia species) in Anglonubian goats in the Sultanate of Oman*, Vet Rec, 144 (1999), 296–297.

[11] C. Ghazaei, *Evaluation of therapeutic effects of antihelminthic agents albendazole, fenbendazole and praziquantel against coenurosis in sheep*, Small Ruminant Res, 71 (2007), 48–51.
[12] J. M. Gonzalo-Orden, A. Díez, J. R. Altónaga, J. M. Gonzalo, and M. A. Orden, *Computed tomographic findings in ovine coenurosis*, Vet Radiol Ultrasoun, 40 (1999), 441–444.
[13] B. E. D. Hago and M. T. Abu-Samara, *A case of Multiceps gaigeri coenurosis in a goat*, Vet Parasitol, 7 (1980), 191–194.
[14] M. B. Ing, P. M. Schantz, and J. A. Turner, *Human coenurosis in North America: case reports and review*, Clin Infect Dis, 27 (1998), 519–523.
[15] J. Kaufmann, *Parasitic Infection of Domestic Animals*, Birkhäuser, Berlin, 1996.
[16] A. Komnenou, S. Argyroudis, N. Giadinis, and A. Dessiris, *Surgical treatment of coenurosis (gid) in sheep*, Vet Rec, 147 (2000), 242–244.
[17] T. Negita and A. Ito, *In vitro hatching of oncospheres of Taenia taeniaeformis using eggs isolated from fresh, frozen, formalin-fixed and ethanol-fixed segments*, J Helminthol, 68 (1994), 271–272.
[18] M. Nooruddin, A. S. Dey, and M. A. Ali, *Coenurosis in Bengal goats of Bangladesh*, Small Ruminant Res, 19 (1996), 77–81.
[19] P. Popesko, *Atlas of Topographical Anatomy of the Domestic Animals*, W. B. Saunders Co., Philadelphia, PA, 4th ed., 1984.
[20] P. Popesko, B. Hájovská, V. Komárek, F. Marvan, and M. Vrzgulová, *Anatómia hospodárskych zvierat*, Príroda, Bratislava, 1992.
[21] T. C. Price, M. H. Dresden, T. Alvarado, J. Flanagan, and C. L. Chappell, *Coenuriasis in a spectacled langur (Presbytis obscura): praziquantel treatment and the antibody response to cyst antigens*, Am J Trop Med Hyg, 40 (1989), 514–520.
[22] A. Scala, G. M. Cancedda, A. Varcasia, C. Ligios, G. Garippa, and C. Genchi, *A survey of Taenia multiceps coenurosis in Sardinian sheep*, Vet Parasitol, 143 (2007), 294–298.
[23] D. Sharma and P. Chauhan, *Coenurosis status in Afro-Asian region: A review*, Small Ruminant Res, 64 (2006), 197–202.
[24] G. C. Skerrit, *Coenurosis*, in Diseases of Sheep, W. B. Martin and I. D. Aitken, eds., Blackwell Scientific Publications, Oxford, 2nd ed., 1991.
[25] G. C. Skerrit and M. F. Stallbaumer, *Diagnosis and treatment of coenurosis (gid) in sheep*, Vet Rec, 11 (1984), 399–403.
[26] E. J. L. Soulsby, *Helminths, Artropods and Protozoa of Domesticated Animals*, Bailliere Tindall, London, 1982.
[27] Y. Takemoto, T. Negita, K. Ohnishi, M. Suzuki, and A. Ito, *A simple method for collecting eggs of taeniid cestodes from fresh, frozen or ethanol-fixed segments*, Int J Parasitol, 25 (1995), 537–538.
[28] M. Tirgari, B. R. Howard, and A. Boargob, *Clinical and radiographical diagnosis of coenurosis cerebralis in sheep and its surgical treatment*, Vet Rec, 120 (1987), 173–178.
[29] F. Toofanian and B. Ivoghli, *Cerebral coenurosis in a wild sheep (Ovis ammon)*, J Wildl Dis, 12 (1976), 550–551.
[30] G. M. Urquhart, J. Armour, J. L. Duncan, and F. W. Jennings, *Veterinary Parasitology*, Blackwell Publishing, Edinburgh, 2nd ed., 1996.
[31] A. Varcasia, G. Tosciri, G. N. Coccone, A. P. Pipia, G. Garippa, A. Scala, et al., *Preliminary field trial of a vaccine against coenurosis caused by Taenia multiceps*, Vet Parasitol, 162 (2009), 285–289.
[32] W. D. Vink, C. M. Lopes Pereira, A. Nota, and K. I. M. Balogh, *Prevalence of coenuriasis in goats in Tete Province, Mozambique*, Ciencia e Tecnica Veterinaria, 3 (1998), 29–34.
[33] I. C. Wang, Y. X. Ma, C. H. Kuo, and P. C. Fan, *A comparative study on egg hatching methods and oncosphere viability determination for Taenia solium eggs*, Int Journal Parasitol, 27 (1997), 1311–1314.
[34] J. M. Willis and I. V. Herbert, *A method for estimating the age of coenuri of Taenia multiceps recovered from the brains of sheep*, Vet Rec, 121 (1987), 216–218.

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Effect of age and gender in the pharmacokinetics of albendazole and albendazole sulphoxide enantiomers in goats

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ABSTRACT

The kinetics of albendazole metabolites and albendazole sulphoxide enantiomers were studied in 2- and 14-month-old female and male goats, after a single oral dose administration (10 mg/kg) of an albendazole formulation. Blood samples from the jugular vein were collected at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, 48 and 54 h post-treatment and analyzed using a high performance liquid chromatography method. In all groups the area under the plasma concentration-time curve (AUC) and peak concentration (C_{max}) values of (+)-ABZSO were significantly higher than those of (−)-ABZSO. The AUC and C_{max} values obtained for (+)-ABZSO and (−)-ABZSO in adult animals were higher compared to the results in young animals, showing significant differences except for (+)-ABZSO in female animals. In young animals, independently of gender, the C_{max} appeared earlier compared to adult animals. The mean residence time (MRT) values were shorter in young animals compared to adult animals for all compounds analyzed. No sex-related differences were found for any of the parameters calculated except for the (+)-ABZSO from adult animals.

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Short Communication

Efficacy of albendazole against *Taenia multiceps* larvae in experimentally infected goats

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ABSTRACT

A controlled trial was conducted to evaluate the efficacy of three therapeutics regimes of albendazole (ABZ) against *Taenia multiceps* larvae in experimental infected goats. Forty-nine goats experimentally infected with 3000 *T. multiceps* eggs were selected and randomly divided into treatment or control groups. Treatment with 10 mg/kg for 3 days for group 1 (G1), 10 mg/kg for group 2 (G2) and 20 mg/kg/day for group 3 (G3) was applied 2 months after infection; group 4 (G4) served as a control group. A treatment with doses of 10 mg/kg/day for 3 days on group 5 (G5) and group 6 (G6) was used as control, 5 months after the infection. The efficacy of ABZ was assessed as percentage of non-viable cysts which were determined by morphologic characteristics, movement and methyl blue staining technique. The efficacy of ABZ against 2 months old cysts was significantly different from the control and were 90.3% (28/31), 72.7% (8/11) and 73.9% (14/19) for G1, G2 and G3, respectively. No differences were observed in cyst viability between treated and control groups for 5-month old cysts. The results in this study indicate that ABZ is effective in goats against 2-month-old cysts of *T. multiceps* larva located in tissues outside the brain.