Anthelmintic and acaricide resistance in small ruminants of North West Province, South Africa

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DEDICATION

To my Heavenly Father,

‘Never be afraid to trust an unknown future to a known God’

‘To the late Mr. Paulus Isaac Emsley and the late Dr. Makhosazana Yvonne Motloang, thank you for teaching me that there are no shortcuts to any place worth going’.

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ABSTRACT

In South Africa, anthelmintic and acaricidal resistance is a growing problem and poses a real economic threat to communal livestock raised by rural communities. The development of new anthelmintic and acaricidal drug products is exceptionally costly, so it is important to trace errors that can be reversed to maximize production in rural small-scale farming. This study aimed to assess the level of anthelmintic and acaricide resistance in small stock in the North West province of South Africa. A questionnaire survey was conducted to assess treatment strategy and farm management practices to 86 small- scale farmers in North West province. Results indicated that small-scale farmers (89%) relied solely on the use of anthelmintics, while 11% did not practice any form of worm control practice. Worm infection was ranked the second most important constraint of productivity in livestock as compared to ticks. Most farms that used anthelmintics preferred Benzimidazoles (BZD) (89%), Oxytetracycline (OXY) (78.14%), Levamisole (LEV) (18%) and Macrocyclic lactones (ML) (3.44%). Generally, 58.97% of farmers treated 2 times a year. Treatments in most farms depended on visible clinical signs not the epidemiology of parasites. The most common risk factor associated with the occurrence of resistance in both districts was the use of anthelmintics without weighing the animals to determine the correct dosage. Limited farming experience was also shown as one of the risks based on the questionnaire results.

The faecal egg count reduction test (FECRT%) was used to assess the development of anthelmintic resistance (AR) of BZD, LEV, and ML in sheep and goats of small holder farmers. Anthelmintic efficacy of 50% was considered as the threshold for development of AR. No significant difference was shown in nematode egg count after 14 days ($p=0.380$). High levels of AR development, particularly against BZD was detected. Unprecedented levels of AR appear to be enabled by under/overdosing and lack of drug rotation.

Egg hatch assay (EHA) and larval mortality assay (LMA) was used to determine AR at a discriminating dose (DD) of 0.1 µg/ml TBZ. The EHA and LMA results showed development of AR against TBZ in all districts. A strong correlation existed between FECRT, EHA, and LMA as tests confirmed the occurrence of AR in all the districts identified. Resistant nematodes after treatment were confirmed using polymerase chain reaction (PCR) targeting the Internal transcribed spacer 2 (ITS2) gene using genus specific primer pairs. PCR detected the presence of *Haemonchus* spp. and *Oesophagostomum* spp post AR tests. *Haemonchus* spp. was identified by coproculture, and PCR tests as the most dominant resistant nematode genera.

Acaricidal resistance (ACR) against fluazuron 2.5% and flumethrin 1% (Drastic Deadline extreme®) (DDE) pour on was assessed using adult immersion test (AIT) on *Rhipicephalus evertsi evertsi*. Results obtained on trial showed high efficacy (>99%) against the ticks. Oviposition was inhibited, indicating ability of fluazuron 2.5% and flumethrin 1% to inhibit tick oviposition reproductive parameters of ticks. In both districts, this study reported the presence of ACR to GIN infections on sheep and goat farms, but no ACR was detected against the currently used acaricide.

Key words: Anthelmintic resistance, Acaricidal resistance, Gastrointestinal nematodes, Egg hatch assay, Larval mortality assay, Adult immersion test, North West, South Africa.

ABBREVIATIONS

Acaricidal resistance (ACR)

Adult immersion test (AIT)

Anthelmintic resistance (AR)

Benzene hexachloride (BHC)

Benzimidazoles (BZD)

dichlorodiphenyltrichloroethane (DDT)

Dimethyl sulfoxide (DMSO)

Discriminating dose (DD)

Dr Kenneth Kaunda (DRKK)

Dr Ruth Segomotsi Mompati (DRSM)

Drastic Deadline extreme® (DDE)

Egg weight (EW)

Egg hatch assay (EHA)

Eggs per gram (epg)

Faecal egg count reduction test (FECRT%)

Faecal egg count (FEC)

Female mean weight (FMW)

First stage larvae (L1)

Gastrointestinal nematodes (GIN)

Imidazothiazoles (IMID)

Internal transcribed spacer 2 (ITS2)

Larval mortality assay (LMA)

Levamisole (LEV)

Lower confidence limit (LCL)

Macrocyclic lactones (ML)

Moxidectin (MOX).

Organophosphate (Ops)

Oxytetracycline (OXY)

Percentage inhibition of oviposition (%IO)

Polymerase chain reaction (PCR)

Reproductive index (RI)

Thiabendazole (TBZ)

Third larvae stage (L3)

World Association for the Advancement of Veterinary Parasitology (WAAVP)

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CHAPTER ONE

INTRODUCTION

1.1 Background

Communal farming is among the oldest farming systems in the world and is primarily practiced in developing countries by smallholder farmers, particularly in rural areas of African countries (Valbuena et al. 2012). It can be described as an area where agriculture is primarily subsistence-oriented, and rangelands are used to pasture domestic livestock (Masiteng et al. 2003). Department of Agriculture of the Republic of South Africa reported communal farming as one of the sub-sectors of livestock farming contributing immensely to alleviating poverty and diversifying livelihoods (Dixon et al. 2001). Seventy-five percent of rural communities in South Africa depend on livestock, contributing up to 33% of household income (Khapayi and Celliers 2016). The functions of livestock can be classified in several ways, including household use (food in form of milk and meat, fuel, and fertilizer). Livestock also plays an important role religiously and culturally [social status, wedding, and ancestral ceremonies] (Moyo and Swanepoel 2010; Bettencourt et al. 2015). Economically livestock production is an essential component of rural agriculture in southern Africa accounting for roughly 49% of agricultural output and 36% of the population's protein needs (Cheteni and Mokhele 2019). According to Slayi (2014), one of the major problems in communal farming is the continued exposure of various ecto-endoparasites on grazing fields, threatening maximum production.

1.1.1 Endoparasites: Gastrointestinal nematodes

Gastrointestinal nematodes (GIN) infections are widespread in small livestock and are classified into four superfamilies: Trichostrongyloidea, Strongyloidea, Metastrongyloidea, and Ancylostomatoidea (Sissay et al. 2007). Superfamily Trichostrongyloidea is considered the most common parasite responsible for diseases and production loss in small ruminants (Sissay et al. 2007). The most importantand widely prevalent nematode

species in the Trichostrongyle group are *Haemonchus contortus, Trichostrongylus colubriformis, Nematodirus battus, Teladorsagia circumcincta, Cooperia* spp*., Nematodirus spathiger, Oesophagostomum* spp*, Bunostomum* (Tariq 2015). Among the mentioned parasites infecting ruminant. gastrointestinal tract, *T. circumcincta, H. contortus,* and *Trichostrongylus* spp. are the most common problematic nematodes (Roeber et al. 2013). However, the prevalence is primarily influenced by factors including pasture adaptation, temperature, humidity, and host grazing behaviour. Optimum temperature suitable for development of nematode species is between 22 and 26 °C, along with a humidity of 100 percent (Torres-Acosta et al. 2012). Nematodes are most likely to die in unfavourable environmental conditions (Mphahlele et al. 2021).

Severity of GIN depends on factors such as present parasite species, the number of worms present in the gastrointestinal tract, the host's general health and immunological condition, age, sex, nutritional condition, and environmental factors such as climate and type of grazing pasture (Gunathilaka et al. 2018). For example, some parasites like *Haemonchus* spp. prefer warm, moist weather whereas *T. colubriformis* and *T. circumcincta* require cool or moist weather (O'Connor et al. 2006). In addition, any combination of several factors such as poor nutrition, concurrent disease, stress, overstocking, or pregnancy / lactation may result in a loss of host immunity to parasites. Risk factors attributable to nematode infection, whether farming practices or environmental factors, must therefore be established to introduce innovative approaches to GIN management (Kaplan 2013).

1.1.2 Ectoparasites: Ticks

Ticks are hematophagous terrestrial invertebrates that belong to the phylum Arthropoda which consists of one suborder (Ixodida) that is divided into 3 families, Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae (ticks of both hard and soft tick features) (Nicholson et al. 2019). Family Ixodidae consists of more than 700 described species (Guglielmone et al. 2010), with species from the following genus considered to be of veterinary and medical significance; *Ixodes, Amblyomma, Rhipicephalus, Dermacentor,*

Haemaphysalis, Cosmiomma, Aponomma, Margaropus, Rhipicentor and *Hyalomma* (Mahlobo 2018). Soft ticks from the Argasidae family consist of 193 species described in the following genus: *Argas, Ornithodoros, Otobius, Antricola, Nothoaspis* and *Carios* (Guglielmone et al. 2010). Whilst Nuttalliellidae family consist of one genus and one species called *Nuttalliella namaqua*, for which little is known about its life cycle (Walker 2003; Gray et al. 2013). Various tick species have different preferences when it comes to geographic distribution and seasonal occurrence.

Ticks are important vectors of infectious disease of small ruminants, acting as reservoirs or multipliers of organisms transmitting several major tick-borne diseases. They are one of the world's most important human disease vectors, second to mosquitoes (Wilson et al. 2020). Ticks can be grouped into the following aetiological agents in livestock; protozoal, rickettsial, bacterial, or viral. These diseases are found to be more prominent in the tropical and subtropical regions. It is estimated that tick-borne pathogens are responsible for more than 60 disease cases worldwide in both domestic and wild animals (Sayler et al. 2016). Disease caused by *Ehrlichia ruminantium*, was reported to cost US$ 44.7 million annually for treatment (Mtshali et al. 2015). The global demand for animal health drugs, exceeds that of human medicine with approximately EUR 30 billion annually (Waller 2006). This puts emphasis on requirement for new resistance-breaking preventatives and treatments. Although the most successful methods of endo- and ectoparasite control rely on the use of commercial drugs, knowledge of levels of resistance to commercial drugs used will help to reduce spread of drug resistance.

1.1.3 Problem of development of resistance to anti-parasitic drugs

Management of infectious diseases depend on the continuous use of commercial drugs including anthelmintics, antibiotics and acaricides. Dependency on therapeutic doses of drugs has tremendously decreased the efficacy of these commercial drugs (Varady et al. 2011). Resistance to anthelmintic and acaricidal have been known to be a problem for at least 40 years dating back to the early 1960s (Hingham et al. 2016). In the small ruminant production industry, parasite immunity to therapeutic drugs has increased to become a major economic issue. Across the world, global surveys have reported severe cases of

resistance in countries such as Australia, Latin America, New Zealand, and South Africa (Spickett et al. 2011; Selzer et al. 2020). Gastrointestinal nematodes and ticks are found to primarily affect small ruminants among the groups described (Taylor 2012), and numerous resistance reports have been reported in these parasite classes (Mazhangara et al. 2020).

1.2 Problem statement

Sheep and goats serve as household assets with multiple livelihood functions in smallholder farming systems. Small ruminants can survive better under drought conditions due to their low body mass and low metabolic requirements than cattle which make them ideal or manageable for rural poor farmer's livelihoods (Oluwatayo and Oluwatayo 2012). Despite their hardness, studies have shown that infections caused by ticks and GIN are a major impediment to the global production of small ruminants (Zanzani et al. 2014). Due to the lack of nutrient-dense feed on pasture and the lack of land ownership, livestock husbandry in rural South Africa requires communal grazing in open fields, resulting in different livestock species grazing together increasing risk of contamination. Exposure to infected pastures results in animals being infected with variety of ecto and endoparasites (Idris et al. 2019).

Commercially available insecticides, acaricides and anthelmintics have been utilised to combat parasite infection. Despite the efficacy of these synthetic drugs, endo and ecto parasites continue to pose one of the greatest production problems in livestock (Lyndal-Murphy et al. 2007). The cost of controlling parasites takes a toll on any stock owner particularly in rural communal farms. In developing countries of the African continent, commercial drugs are expensive and sometimes unavailable leading to the use of poor quality or altered products (Luseba and Tshisikhawe 2013). Misuse of these synthetic drugs can result in the development of resistance rendering the products inefficient to eradicate or minimize parasite load. This results in farmers using incorrect doses (either increasing or decreasing) the intensity of the drugs, thus increasing development of resistance.

Studies conducted on rural communal farms indicated that there is a lack of understanding of parasites infesting livestock, particularly helminths (Vatta et al. 2001; Tsotetsi and Mbati 2003; Hlatshwayo and Mbati 2005; Lindberg and Vatta 2006; Maphosa and Masika 2012; Mthi et al. 2017; Mphahlele et al. 2019). Helminthosis is generally underestimated because of the chronic and asymptomatic nature of the infection (Idris et al. 2019). A study by Brown et al. (2013) revealed that small-scale rural farmers in the North West province were knowledgeable about ticks, however, they were not well informed that ticks are vectors of variety of diseases. Most farmers recognized physical damage caused by heavy tick infestation on hides, ears, tail, genitals and mortality in kids and lambs. This can result in farmers misdiagnosing animals due to lack of knowledge regarding diseases affecting small ruminants, thus increasing resistance. Under/overdosing and continued use of one or more classes of anthelmintics or acaricides, regardless of efficacy status, are common factors that contribute to the development of drug resistance (Bakunzi 2003, Besier et al. 2016). Therefore, this study sought to collect information on the knowledge of resource-poor farmers on commercial drug use in selected districts of North West province

1.3 Aim of the study

To assess the level of anthelmintic and acaricide resistance in small stock in North West province of South Africa.

Objectives

- To determine the occurrence of anthelmintic resistance against the three most widely used anthelmintic drug classes in the two districts of North West province using a combination of in vivo, in vitro, and molecular techniques.
- To assess development of acaricide resistance against commonly used acaricide in selected districts of the North West province using adult immersion test.
- To document management and treatment strategy on sheep farms in North West province using a questionnaire survey

Hypothesis

- Gastrointestinal nematodes and ticks infecting small ruminants have developed resistance against all commonly used anthelmintic and acaricidal drugs in the two districts of the North West province
- Management and treatment strategies have impact on development of resistance on strategy on sheep and goat farms in the two districts of the North West province.

1.4 Significance of the study

The increased difficulty to combat GIN and tick-borne infection in ruminants, due to progressing AR and ACR calls for measures that can be implemented to slow down rate of resistance in communal farming systems. The findings of this study will directly benefit farmers by providing strategies that can be implemented to maximize production. The detection of AR and ACR in North West will help outline effective management systems against infections caused by helminth and ticks. It will also be beneficial to farmers in a large scale of animal husbandry, creating a better understanding of sustainable production in communal farming systems in North West.

1.5 Dissertation outline

This dissertation consists of five chapters.

Chapter one - Introduction:

Background to the study, statement of the problem, aim and objectives of the study.

Chapter two - Literature review:

Review of relevant literature in relation to gastrointestinal parasites, ticks, and anthelmintic and acaricide treatment of small stock.

Chapter three - Materials and methods:

Describes the different study areas as well as materials and methods used in the study.

Chapter four - Results:

Presents details of generated data and analyses thereof.

Chapter five - Discussion, conclusion, and recommendations:

Gives interpretation of data generated in this study, concluding remarks and recommendations.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Common types of anthelmintic drugs

Nematode management seeks to ensure that parasite populations do not reach levels that are consistent with economic development (Charlier et al. 2014), thus anthelmintic drugs are used to maximize production. Small-scale farmers in South Africa depend heavily on the use of anthelmintic drugs to manage GIN (Burke and Miller 2020). Throughout veterinary medicine, anthelmintic drugs are of great importance because of their capability to remove the immature and mature stages of almost all considered large GIN (Zajac and Garza 2020). Since the introduction of broad-spectrum drugs, management of parasites has depended heavily if not exclusively on chemotherapy. Anthelmintic drugs can be classified into three groups: BZD, ML and imidazothiazoles (IMID) (Table 2.1). Resistance of parasites to the available drugs has however, become a challenge, which requires development of new drugs using a different mode of action compared to previous anthelminthic drugs (Chen et al. 2010)

Table 2-1: Classes of commonly used anthelmintic drugs globally

Acronym	Drug class	Drugs	Trade names
BZD	Benzimidazoles	Fenbendazole, Albendazole, Oxybendazole	SafeGuard, Panacur, Valbanzen, Synanthic
IMID, TETR	Imidazothiazoles and Terahydropyrimidine	Levamisole, Morantel, Pyrantel	Prohibit, Rumatel, Positive Goat Pellet,Strongid
ML	Macrocyclic lactone (Avermectins and Millbimycins)	Ivermectin, Doramectin ,Eprinomectin, Moxidectin	Ivomec, Eprinex, Dectomax, Cydectin,Quest

2.1.1 Benzimidazole

Benzimidazoles group which is also known as “white wormers” is the oldest class of anthelmintics authorized and is considered as one of a large family of anthelmintic drugs with a wide range of activity against GIN parasites (Gomes and Nagaraju 2001). In 1962 TBZ was the first anthelmintic drug to be developed from this class (Gomes and Nagaraju 2001). Several similar compounds were synthesized after the discovery of TBZ, which are the most used drugs in small ruminants including fenbendazole and albendazole (Valbantel® and Valbazen® Ultra) (Dilks et al. 2020). Bakunzi (2008) reported resistance to these synthesized compounds with an efficacy of 68% and 58%, respectively indicating high resistance levels. In South Africa, resistance to the BZD community has been recorded and the results indicate that the resistance rate is still increasing alarmingly (Ramotshwane 2011; Bakunzi et al. 2013; Tsotetsi et al. 2013; Mphahlele et al. 2021).

2.1.2 Imidazothiazoles

Imidazothiazoles are group anthelmintics which were first discovered in 1966 (Kamal et al. 2013) and this group is further divided into 2 groups, namely, LEV (LEV; Prohibit®, and Levasol® and Tramisol®) and terahydropyrimidine (Pyrantel, Morantel, and Oxantel salts) (Keeton 2016). Levamisole is the anthelmintic drug most widely used in this group, although all of them have the same mode of action (Keeton 2016). It is available in combination with BDZ for control and treatment of sheep nematode. In addition to being used as anthelmintic drug for livestock, it has also been used to treat various human diseases, such as influenza colon, head, and neck cancer (Kamal et al. 2013). It acts by triggering nicotinic acetylcholine receptors resulting in worm paralysis that is subsequently washed out of the host intestine by peristalsis (Keeton 2016). According to Enejoh and Suleiman (2017) absorption of the drug is via a trans-cuticular mechanism. The LEV group has a wide range of activity and is effective against many larval stages, but not arrested larvae (Elfawal et al. 2019) and has a narrow margin of safety compared to other anthelmintics, although toxicity is usually the result of excess dosage.

2.1.3 Macrocyclic lactones

This group of drugs consist of avermectins such as ivermectin (IVM) like drugs and milbemycin's such as moxidectin (MOX). The first anthelmintic drugs of ML were discovered in 1973, followed by the discovery of milbemycin in 1975 (Prichard and Gaery 2019). Moxidectin is the third avermectins to be discovered at the end of the 1980s. Ever since 1981 onwards, IVM had been approved for use in cattle, goats, horses, swine, dogs, camels, reindeer, bison, and humans in more than 60 countries (Prichard and Geary 2019). Resistance to this class was reported by Sezler and Epe (2020) in *Trichostrongylus* spp*., Cooperia* spp., *Nematodirus* spp., and *Teladorsagia* spp. in sheep and goats. Besides the broad-spectrum BZD, IMID and IVM drugs other smaller groups such as mid- spectrum organophosphates, narrow spectrum halogenated salicylanilide were and are still used as nematocidal anthelmintics although they have limited activity against different stages and species of helminths (Abongwe et al. 2017).

2.2 Factors contributing to anthelmintic resistance

The term AR is defined as the ability of parasites to become susceptible to normal dosages of a drug and does not promote a consistent reduction of worms or released eggs (Chan et al. 2018). Resistant worms survive and transmit their resistant gene resulting in failure of treatment and this characteristic is hereditary and selected for, because the recipients transfer the resistant gene on to their offspring (Choi et al. 2017). Such resistant genes are initially uncommon throughout the population or appear as unusual mutations, but as selection progresses, their population proportion increases, as does the resistant parasite proportion (Mphahlele et al. 2019). It is essential to identify major factors that promote AR to develop appropriate measures to combat it (Amulya et al. 2016).

Habitually AR is the first to be questioned in obvious anthelmintic failure cases. However, according to Amulya et al. (2016) different factors can be accountable for lack of drug efficacy, such as:

- failing to ensure that all animals are given the correct dosage, by not using the

correctcalibration of drench guns

- administering medication without using the proper injectable drugs, needle size, formulation, or injection site (to prevent abscess formation).
- underdosing, treatment frequency, and timing of treatment also contribute to resistance.

2.2.1 Underdosing

Underdosing is when anthelmintic drugs are inadequately administered, which allows survival of heterozygous resistant parasites accelerating the development anthelmintic resistance after treatment (Shalaby 2013). Research conducted by Bakunzi (2003) and Tsotetsi et al. (2013) on AR of nematodes in communally grazed goats in South Africa reported underdosing as a risk factor for AR development caused by visual measurement of animal weight as opposed to actual weight before dosing to decide the appropriate anthelmintic dose. It is important to measure the animal's weight to determine the appropriate dosage ensuring that treatment is fully effective (Less et al. 2002).

In addition, bioavailability differences in different host species are also critical in deciding on the correct dose. Bioavailability refers to the degree and rate at which a drug is absorbed in the circulatory system of an animal (Hetal et al. 2010). Dyary (2016) reported that BZD and LEV are absorbed faster in sheep than goats, thus goats are given approximately twice the dose given to sheep. Due to shortage of drugs specifically for goats, most licensed anthelmintic drugs for sheep are frequently used in goats without optimizing dosage regimens and, determination of pharmacodynamics and pharmacokinetics (Aksit et al. 2015).

2.2.2 Treatment frequency

Numerous studies and investigations on AR have shown that high frequency of drug treatment of a whole flock increases the selection pressure to produce AR during the preparatory phase of the nematode life cycle (Vatta et al. 2001; Sargison et al. 2007; Hernández-Villegas et al. 2012). Since the commercial anthelmintics were introduced,

farmers relied on regular treatment with ineffective medication to control GIN in their flocks. Treatment rates have increased due to demand of livestock production, which in turn results in extensive frequent drenching of anthelmintic drugs of all animals in the flock and in many cases consequently selected for severe AR (Tsotetsi et al. 2013). Following the introduction of TBZ in 1961, anthelmintic drug use became a standard, and treatment was routinely given (Campbell 2016). Prichard (1990) was the first to predict in the 1990's that repeated anthelmintic treatment would result in development of resistance. This has been proven to be one of the leading factors contributing to AR as well. A report by Vatta et al. (2001) stated that to ensure good animal health in commercial industry cooperative farms prefer to manage their flocks on continuous dosing with different anthelmintic drugs, which in turn raises the AR level in commercial farming. Therefore, it is necessary for resource-poor farmers not to suffer the same fate as commercial farmers by not complying with the standards of commercial farming.

2.2.3 Timing of treatment

Throughout winter conditions, pastures harbour few infectious larvae, the animals host virtually all the parasite population, which later develop into adult worms that survived anthelmintic treatment. At the beginning of grazing season pastures will be contaminated with larvae laid by resistant worms (Fiel et al. 2017). Therefore, it is important for the ratio between free-living larvae on the field and within the host nematodes to be considered when determining drenching time (Silvestre et al. 2002). A report by Macchi (1997) highlighted that pasture management can decrease the effect of worm infection on livestock. This argument was supported by researchers that speculated that the use of clean pastures increases the selection for resistance, as larval populations on pastures become more and more survivors of the drenching programs. However recently, crop stubble is preferred instead of grazing animals on contaminated pastures (Dyary 2016).

2.3 Occurrence and prevalence of AR

Resistance to anthelmintic drugs in nematodes infecting sheep and goats is a major concern recorded both in commercial and resource-poor farming in many sheep producing

percent development of AR to ML on sheep farms, whilst 37-38% was recorded against BZD (Vlaminck et al. 2018). Similar results of resistance to BZD were recorded in Australia, with 98% prevalence of AR development. In Europe, AR has been reported in the Slovak Republic (Čerñanská et al. 2008), in the UK resistance to BZD, LVM or ML indicated an 82% resistance to all classes. In the northern-western Europe 62%, 50%, 14% showed resistance to the following, MOX, IVM and LVM respectively (McMahon et al. 2017). In the continent of South America multiple resistance to all three drug classes in Argentina flocks was detected, showing a resistance of 50% (Caracostantógolo et al. 2013). Anthelmintic resistance is a serious problem across Africa, especially in South Africa (Mphahlele et al. 2020). Resistance was first established in 1975 and has grown substantially since then (van Wyk and Van Schalkwyk 1990). Three surveys in Mpumalanga, Limpopo and KwaZulu-Natal provinces indicated an average of seventy-nine percent resistance to all three drug classes, against strains *of H. contortus* (Muchiut et al. 2018). Similar results in Limpopo were recorded by a study conducted by Mphahlele et al. (2021). The AR studies still consider this phenomenon as an important issue affecting animal health care to date (Zvinorova et al. 2017; Chitura et al. 2019; Wit and Andersen 2020). Assessing the occurrence of AR is therefore a prerequisite in planning sustainable management strategies and providing solutions to commonly made mistakes that increase levels of resistance.

2.4 Nematodes developing resistance to anthelmintic drugs

The emergence of resistant nematodes threatens sustainable control (Kotze and Prichard 2016). Resistance to all three drug classes of anthelmintic is displayed among trichostrongyloids which mainly includes the following species*: H. contortus*, *T. circumcincta, T. colubriformis*, *O. ostertagi* and *Cooperia* spp. (Mphahlele et al. 2021). The results of respective studies conducted by Tsotetsi et al. (2013) and Mphahlele et al. (2021) reported *Haemonchus* spp. to be the most encountered nematode resistant to BZD, LEV and ML in Gauteng and Limpopo provinces. This is consistent with other studies conducted in South Africa (Carmichael et al. 1987; van Wyk and Malan 1988; Van Wyk et al. 1997;

Vatta et al. 2001; Bakunzi 2008). These results are concurrent to observations of studies conducted in other countries of the African continent such as in Namibia (Kumba et al. 2003), Nigeria (Chiejina et al. 2011); Zimbabwe (Vassilev et al. 2017) and Botswana (Rambu et al. 2020).

2.5 Ticks and tick-borne diseases

Ectoparasites occur permanently (mites and lice) or occasionally during feeding (ticks and flies) on the body of small ruminants (Hopla et al. 1994) and play a vital role in transmitting a variety of pathogens. Among the mentioned ectoparasites, ticks have been reported as the most notorious threat to small stock production (Hopla et al. 1994). In both tropical and subtropical regions, approximately 80% of livestock are affected by ticks and tick-borne disease (Yawa et al. 2020). Anaplasmosis, babesiosis, and theileriosis are the most common diseases transmitted by these pathogens. They are known to cause major problems such as anemia, loss of teats and lameness due to abscesses, as well as myiasis (Waruiru 1998), causing mortality and major depressions in livestock production (Lew-Tabor et al. 2016).

2.6 Management practices to control ticks in livestock

According to Goerge et al. (2004), the use of acaricides is the primary and most effective method used to manage ticks all over the world since the 20th century. Application of acaricides was primarily enforced by government agencies during colonial era in Africa in the late 1900s to early 80s, due to outbreak of tsetse flies (Eisler et al. 2003). This strategy has been seen to be successful in countries like Nigeria, Uganda, South Africa, and Zimbabwe in reducing the incidence and extent of diseases (Eisler et al. 2003).

Method of application include dipping, race spray, hand spray, pour-on, and hand dressing injection and intra-ruminal boluses. Most commercial and small-scale farms use the dipping method, also known as the plunge dip or dip tanks (Moyo and Masika 2009). In South Africa chemical control of ticks began in 1893, when the use of arsenic acaricides was approved (Matthewson and Baker 1975; Drummond 1976). Since then, after rigorous

research, synthetic acaricides such as chlorinated hydrocarbons, carbamates, organophosphates, carbamates, pyrethroids, and formamide have been produced (Montuori et al. 2015). All these acaricides have various modes of action (Table 2.2) (Abbas et al. 2014).

Table 2-2: Commonly used acaricides, their site of action and mode of action

Class of acaricides	**Commonly used acaricides**	**Site of action**	**Mode of action**	**Reference**
Orgnochlorides	Lindane & dieldrin	Nervous system	GABA-gated chloride channel antagonists	Lawrence and Casida (1983)
Organophosphates	Coumaphos & diazinon	Nervous system	Acetylcholine esterase inhibitors (irreversible)	Li et al. (2004)
Carbamates	Carbaryl	Nervous system	Cholinesterase inhibitors	Li et al. (2005)
Pyrethrins/pyrethroids	Cypermethrin & permethrin	Nervous system	Sodium channel modulators	Narahashi (1971)
Formamidines	Amitraz	Nervous system	Octopamine agonists	Chen et al. (2007)

2.6.1 Arsenic dips

Arsenic is a water-soluble inorganic acaricide used for the management of ticks in livestock (Mekonnen 2006). Arsenic compounds were discovered towards the end of the 19th century (Thomas and Troncy 2009). The first record was reported in South Africa in 1983 and was used in the successful eradication program to control *Rhipicephalus (B.) annulatus*, *R. (B.) microplus* and cattle fever from the US (Chaudhuri and Naithani 1964). Owing to evolution of resistance of ticks, environment toxicity and concerns about harmful residues in animal tissue, arsenic compounds are no longer used in the industry and were promptly replaced by organic synthetic insecticides (Bentley and Chasteen 2002).

2.6.2 Organochlorine insecticides

The first synthetic organic insecticides manufactured were organochlorine insecticides (Peter and Cherian 2000) and are considered efficient with their mode of action been by altering sodium ion channels, affecting tick nerve conduction (Chrustek et al. 2018). The first to be used as acaricides on this group of chemicals were dichlorodiphenyltrichloroethane (DDT) and benzene hexachloride (BHC), followed by chlordane, toxaphene, endrin, aldrin, endosulfan, and methoxychlor (Matsumura 1985). Level of toxicity in organochlorine is not very high, however they are persistent in the environment and are prone to accumulate in body fat of animals hence they were phased out of use (Spickett 1987). Residues of compounds such as DDT, endrin and dieldrin are still reported and are still traced in wildlife and water bodies (Spickett 1987).

2.6.3 Organophosphate

The synthesis of organophosphate (OPs) acaricides was primarily for the control of *Boophilus* ticks resistant to organochlorine. Organophosphate acaricides are chemical compounds formed by the alcohol and phosphoric acid reactions (Mekonnen 2006). This class of chemicals is broken down into many forms; however, phosphates and phosphorothionates are the two most common types. Organophosphate and carbamates are comparable in terms of function (Martín-Reina et al. 2017). The mode of action of these insecticides is by inhibiting cholinesterase which is responsible for breaking down acetylcholine (Hemingway and Georghiou 1983). Inhibition of acetylcholine results in over stimulation of the nervous system, destroying transmission of nerve signals in ticks and ultimately resulting in death (Hemingway and Georghiou 1983). The insecticide, however, is most effective when administered at low concentration using appropriate dip plunge. Due to its accumulation in tissues and milk, organophosphate compounds are not to be used in lactating animals (De Deken et al. 2014). Organophosphates are less persistent and less toxic than organochlorine. Thus, to date OP’s are still utilized in agriculture, veterinary practices, and tick management and are sometimes combined with other acaricide groups, primarily pyrethroids (Akre 2016).

2.6.4 Carbamates

Carbamates (e.g., carbaryl, imiprothrin and carbofuran) are derived from carbamic acid and are like organophosphate insecticides structurally and mechanistically (Pohl et al. 2018). Carbamates constitute a versatile class of compounds used as insecticides, miticides, molluscicides, fungicides, and herbicides (Pohl et al. 2018). It is understood that they are less persistent and harmful but continuous use has led to cross-resistance, which can be characterized as conferring resistance to various carbamate drugs by a single resistance mechanism. This has been a problem particularly in some countries of Africa, Australia, and some parts of the US (Moyo and Masika 2009).

2.6.5 Foramidines

This group is also known as amidines, consisting of 2 active compounds amitraz and cymiazol (Hollingworth 2003). They were discovered in 1970 and are still being used for management of ticks in all major cattle production areas in South Africa and other parts of the world. The first field trial with amidines was performed in South Africa which resulted in the compound being named ‘tick detaching acaricides’ because ticks detached after exposure) (Nyoka 2017). Residual activity of these insecticides lasts for 7-10 days. Within 30-60 minutes of exposure the ticks begin to get distressed and disengage their mouthparts from the host. Amidines also decreases proliferation by inhibiting reproduction in arthropods (Jonsson and Hope 2007).

2.6.6 Pyrethroids

The pyrethroids are derived from chrysanthemum family, a class of active synthetic insecticides derived from natural pyrethrin (Davies et al. 2010). This group of insecticides are considered more effective than organochlorine, organophosphate, carbamate and against a broad range of economically important pests. According to Gillingwater et al. (2010), pyrethroids are also used against tsetse flies in areas where animal trypanosomiasis is prevalent such as KwaZulu-Natal province. Pyrethroids are neurotoxic like organophosphates and carbamates (Ongono et al. 2020). The first active chemicals to

be on the market from this group include permethrine and fenvalerate (Davies et al. 2007). In South Africa registered members of this group that are still utilized include cypermethrin, flumethrin, deltamethrin, cyfluthrin, cyhalothrin and alphamethrin (Thacker 2002). Some of these active ingredients are used in conjunction with other acaricides, especially organophosphates. As effective as these compounds are, it has been reported by Jonsson and Piper (2007) that they are expensive, and that ACR develops rapidly as compared to permethrin. In addition, they are also harmful to fisheries and other aquatic species.

2.7 Factors influencing the rate of emergence of acaricidal resistance

The rate at which resistant alleles are formed in a population depends on the prevalence of the original mutation population prior to treatment, mechanism behind inheritance of resistant allele, the ratio of ticks exposed to acaricides and dispersal of resistant ticks to a new population (Knolhoff and Onstad 2014). Genetic resistance is hereditary and spread through reproduction of resistant individuals (Knolhoff and Onstad 2014). Heterozygous individuals in a population are selected by acaricidal pressure resulting in homogenous resistant population (Knolhoff and Onstad 2014).

Ticks can also be naturally resistant to acaricides, due to reduced penetration through integument or reduced permeability of the chemical rendering the insecticide ineffective (Hedimbi 2009). Natural resistance is the product of natural selection, whereby in a population certain tick species are biochemically or physiologically adapted to combat the effects of acaricides (Andersen and Rathmell 2015). According to De Deken et al. (2014), adaptation to resistance is not because of exposure to acaricides but the resistant genotype population present. Therefore, whether acaricides are applied in low or high concentration dosage not all ticks exposed die, some survive and may develop resistance. Other attributes related to acaricide resistance include application methods, treatment intervals, acaricide concentration, mixtures of rotation of acaricides, and ecological niches (Kunz and Kemp 1994).

2.8 Occurrence of acaricidal resistance in ticks

Resistance to acaricides poses a major threat to livestock production, as these chemicals are an ever-declining resource, and the discovery of new chemicals is costly and thus prohibit development (Nolan 1990). In the African continent, Ethiopia and South Africa recorded losses of over US$ 25 million and US$ 487 million, respectively, due to tick-borne diseases (Adam 2019). Acaricidal resistance levels are greater in developed countries, with losses of more than US$3 billion in the US (Yawa et al. 2020). According to Baker (1982), South Africa's use of acaricides is likely to remain for the foreseeable future, however, they are used by livestock producers who can afford the cost of the drugs. The continual use of these synthetic drugs has led to drawbacks such as residues in milk and meat produce and development of resistant tick strains (Willadsen and Kemp 1988).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical clearance

The research was approved by the Integrated Pest Management Scientific Committee, North-West University, and the Research Ethics Committee of the Faculty of Natural and Agricultural Sciences with reference no: NWU-01948-19-A9. Permission to collect study samples was granted by participating resource-poor farmers with aid of Animal Health Technician of Department of Agriculture, Forestry and Fisheries in both studied districts of the North West province.

3.2 Study area

This research project was carried out on selected small-scale farms in Dr Ruth Segomotsi Mompati (DRSM) and Dr Kenneth Kaunda (DRKK) districts of the North West province (Figure 3.1). Participating famers were recruited with the assistance of Animal Health Technicians in the respective districts.

3.3 Questionnaire survey

To obtain information on livestock management and worm control practices, 86 questionnaires were distributed to 46 and 41 small-scale farmers in DRSM and DRKK, respectively, in the North West province. The questionnaire consisted of two sections. The first section was related to socio-economic profile, animal husbandry and farm management. The second part was dedicated to knowledge regarding helminth parasite control, visible clinical signs caused by parasites,treatment with anthelmintic, anthelmintic products used, dose determination, and mode of application (Appendix 1).

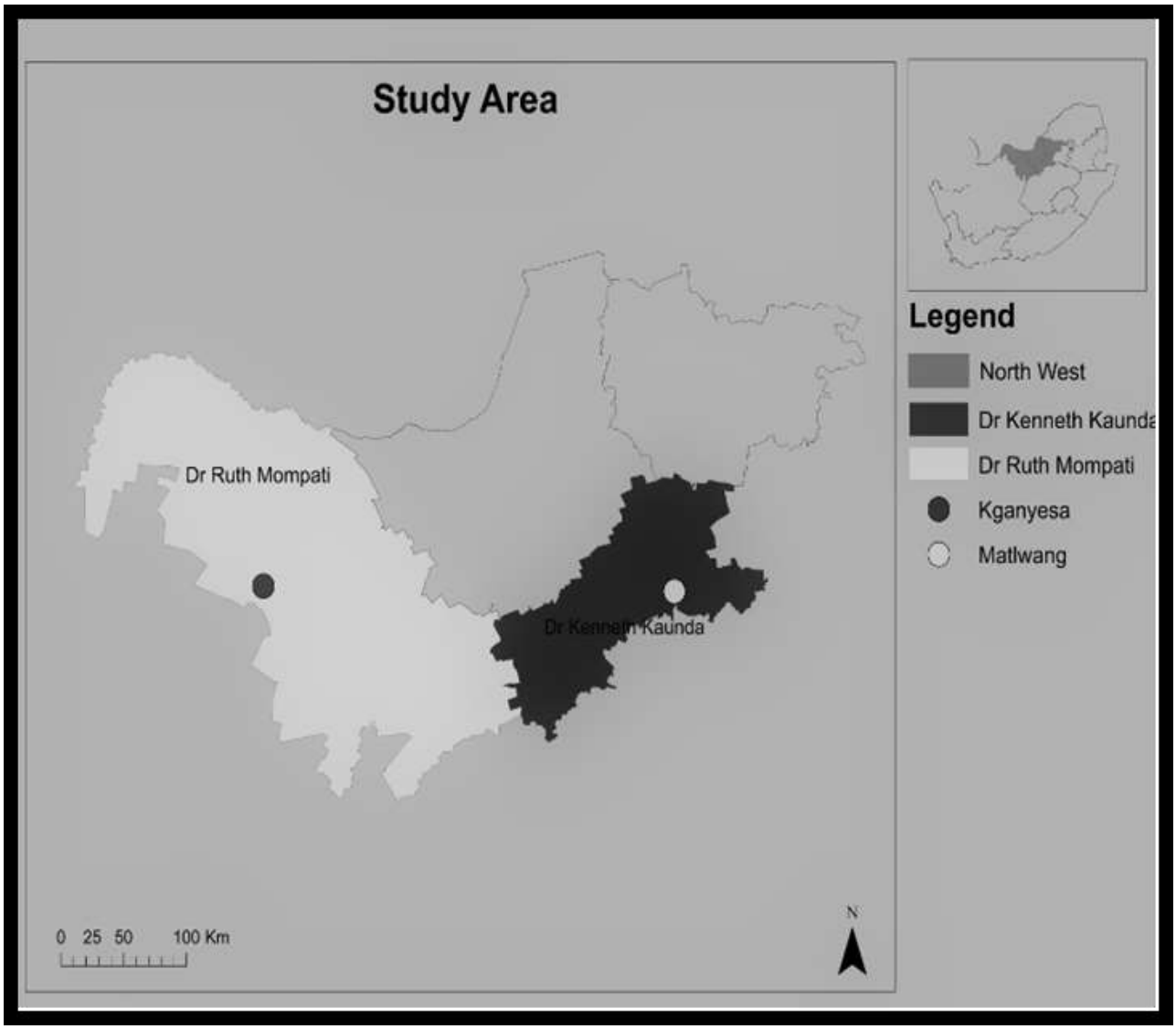

Figure 3-1: Map of North West province showing Dr Segomotsi Mompati and Dr Kenneth Kaunda districts where sampling was conducted. (Map constructed using ArcGIS V10.2.2).

3.4 Anthelmintic resistance

To determine the occurrence presence of AR development, the FECRT% was conductedas described by Coles et al., (2006). Faecal samples were collected directly from the rectaof 249 small ruminants (Figure 3.2) (n = 130 sheep, and n = 119 goats) in Vragas, Kgogojane, Matlwang, Taung and Potchefstroom College of Agriculture in summer, between months of November 2019 and March 2020. Upon collection, samples were transported to the

Parasitology Laboratory of North-West University in a cooler box for analysis. Individual nematode egg counts for each animal were determined by a modified McMaster technique (Reinecke 1983).

3.4.1 McMaster technique (Reinecke 1983)

Faecal samples were weighed, and 2 g was added to 40% sugar solution as a flotation medium. Samples were thoroughly crushed until homogenized using a spatula spoon. The solution was then filled in a two chamber McMaster slide and left to sit for 2 min for eggs to float on the surface of the slide. A light microscope (Nikon Eclipse E100,Japan) was used to determine eggs per gram (epg), by counting the number of eggs perchamber and multiplying by 100. The helminthology atlas by van Wyk et al. (2004) was used to identify the egg genera.

3.4.2 *In vivo* assay: Faecal egg count reduction test

Farm animals with a faecal egg count (FEC) of less than 1000 epg were excluded from AR experiment, as suggested by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline (Coles et al. 2006). Taung and Potchefstroom College of Agriculture were among the farms that were excluded. The remaining 165 animals (75 sheep and 90 goats) from Kganyesa (Vragas and Kgogojane) in DRSM and Matlwang in DRKK were included in the AR trial/experiment. The selected animals had not been treated with any anthelmintic drugs for at least 8 weeks prior to the study.

Figure 3-2: Collection of faecal samples from the rectum of a sheep

Approximately 10 animals per group were allocated to 3 separate treatment groups per farm, and the remaining animals in the flock formed a control group according to the number of animals present per farm (Table 3.1). Prior to treatment, animals were weighed, ear tagged, and faecal samples were collected and analysed using McMaster to determine FEC prior to treatment. Thereafter, animals were treated with recommended doses ML (Ivomec®, Merial, 0.2 mg/kg, injection), LEV (Tramisol Ultra®, Coopers and Intervet, 5

mg/kg, orally) and BZD (Valbazen®, Pfizer, 7.5mg/kg, orally). The control group was not treated. Fourteen days after treatment, faecal samples were collected again directly from the recta, from both treated and untreated animals and analysed using McMaster for FEC post treatment. The faecal egg count reduction percentage was calculated using the formula of Kochapakdee et al. (1995) foreach individual animal

$$(FECR\%) = 100 \times (1-[FEC2/FEC1])$$

Resistance is present if FECRT% is less than 95% and the lower confidence limit is below 90%. If only one condition has been met, then resistance is only suspected.

Table 3-1: Number of treatment and control animals

District	Farm	Animal group	Anthelmintic drug	No of animal	Control
DRSM (n=59	1	Sheep	BZD	10	15
	2	Sheep	ML	10	
	3	Goat	LVM	12	12
DRKK (n=86)	4	Sheep	ML	10	10
	5	Sheep	BZD	10	10
	6	Goat	ML	11	10
	7	Goat	BZD	10	5
	8	Goat	ML	10	5
	9	Goat	LVM	10	5

BZD = Benzimidazole (Valbazen®); LEV = Levamisole (Tramisol Ultra®); ML = Macrocyclic Lactones (Ivomec®)

3.5 *In vitro* assays

The *in vitro* assay conducted included, egg hatch assay and larval mortality which were conducted to determine anthelmintic activity of TBZ. However, before the assays could be conducted, nematode eggs were recovered from faecal samples and screened hence the egg recovery assay was conducted as described by Maphosa et al. (2010).

Fresh faecal samples were transferred into a 50 ml tube and homogenized with water, by breaking pellets until a liquid suspension was obtained. The slurry suspension was filtered through sieves of 117, 70 and 25 µm. The eggs obtained in the 25 µm mesh sieves were back washed with distilled water, then transferred to a 50 ml centrifuge tube and allowed to stand for 1-hour to sediment. The supernatant was carefully discarded, while the sediment was suspended in 40% sugar solution, so that the eggs can float on the surface. The suspension was then poured in another set of tubes and back washed through the 25 µm mesh sieves with distilled water. On a microscope slide, a drop of the extracted suspension was placed and examined under a stereomicroscope to verify the presence of eggs. Liquid containing nematode eggs was then poured into a beaker and sedimentation was tolerated for 2 hours. The concentration of eggs was estimated by counting the number of eggs in 2 aliquots of 0.5 ml of the suspension in a microscope slide repeatedly, and the mean number of eggs per 0.5 ml was determined

3.5.1 Egg hatch assay

Preparation of stock solution was made by dissolving 50 mg TBZ in 5 ml of dimethyl sulfoxide (DMSO) making 10 mg TBZ per ml. Stock solution (1 ml) was dissolved by adding 9 ml of DMSO making 1 mg TBZ per ml. Seven serial dilutions of TBZ ranging from (0.01 µg/ml to 0.5 µg/ml) were added to the 24 well microtiter plate (Table 3.2) (vonSamson-Himmelstjerna et al. 2009). Eggs contained in the egg suspension of 0.5 ml was counted using a dissecting microscope, approximately 100 eggs were pipetted into well of the experimental plate with 10 µl of TBZ solution from each dilution. Ten microliters of DMSO were added in control wells. Plates were sealed to prevent drying out and incubated at 27°C for 48 h; thereafter a drop of Lugol's iodine solution was added to each well to stop further hatching. All tests were replicated three times. The ovicidal activity was expressed basedon the percentage of eggs that failed to develop and hatch. The number of unhatched eggs and the first stage larvae (L1) present per well were counted using a dissecting microscope. Inhibition percentages were calculated using a formula described by Cala etal. (2012):

$$E = \frac{(Eggs + L_1) - L_1}{Eggs + L_1} \times 100$$

Table 3-2: Preparation of thiabendazole solutions

Volume B (µl)	Volume DMSO (ml)	TBZ concentration in workingsolution (µg/ml)	Final TBZ concentration inwell (µg/ml
20	9.98	2	0.01
50	9.95	5	0.025
100	9.9	10	0.05
200	9.8	20	0.1
400	9.6	40	0.2
600	9.4	60	0.3
1000	9	100	0.5

3.5.2 Larval mortality assay

Larval cultures were prepared as described by van Wyk et al. (2004). *In vitro* cultures from nematode eggs were prepared after collection from microscopically positive sheep faecal samples. Pooled faecal samples from each farm in the study was prepared. Ten grams of faeces were placed in glass jar, then crumbled thoroughly before being mixed with vermiculite chips for air circulation. A hole was left in the centre of culture by placing a stamper in the centre of the jar vertically while the mixture is compacted slightly aroundit. The cultures were moisturized sufficiently with distilled water, incubated for 7 days under humidified conditions of 27˚C; and checked periodically and moistened if necessary. On the 7th day, the L3 larvae were harvested.

Mortality assay was conducted according to the method described by McGaw et al. (2007). Adult L3 was harvested from the *in vitro* cultures prepared as described by van Wyk and Mayhew (2013). The 5 ml of L3 solution were placed in microtiter plate with 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.5 µg/ml working concentration of TBZ as prescribed by von Samson-

Himmelstjerna et al. (2009). Distilled water was used as a control. After the addition of concentrations, larval counts of dead L3 were conducted at (2 h, 24 h, and 48h intervals). All tests were repeated three times. Percentage inhibition of larval development was calculated using the formula described by Coles et al. (1992) and Bizimenyera et al. (2006) with slight changes:

$$\text{Inhibition percentage (\%)} = 100(1-X_1/X_2)$$

3.5.3 Detection of AR using PCR assay

3.5.3.1 DNA extraction from larvae

Detection of resistant nematodes was conducted using conventional polymerase chain reaction (PCR) targeting the internal transcribed spacer 2 (ITS2) gene using genus specific primer pairs (Table 3.3). One reverse universal primer was used for all primer pairs.

Table 3-3: Oligonucleotide primers used to amplify ITS2 gene for identification of resistant nematodes

Primer sets	Forward	Reverse	Fragment size (bp)	Annealing temperature(°c)	References
HAE-	5'CAAATGGCATTTGTCTTTTAG 3'	5'TTAGTTTCTTTTCCTCCGCT 3'	256	55°C	Veena et al. 2020
OEC-	5'TCGACTAGCTTCAGCGATG 3'	5'TTAGTTTCTTTTCCTCCGCT 3'	333	53°C	Veena et al. 2020
TRI-	5'TCGAATGGTCATTGTCAA 3'	5'TTAGTTTCTTTTCCTCCGCT 3'	398	54°C	Veena et al. 2020
TEL-	5'TATGCAACATGACGTACGACGCG 3'	5'TTAGTTTCTTTTCCTCCGCT 3'	218	55	Bott et al. 2009

The prepared HotStar Taq PCR (QIAGEN) master-mixes were performed in a total volume of 25 µl for each PCR assay as depicted in Table (3.4). The PCR reaction was conducted

under the following conditions: initial denaturation at 95°C for 15 minutes followed by 50 cycles of denaturation at 94°C for 1 minute, annealing at 47°C for 1 secondand extension at 72°C for 1 minute, with a final elongation step at 72°C for 1 minute. Following the amplification, 5 µl amplicon was resolved by gel electrophoresis using 1% (w/v) agarose gel stained with ethidium bromide and visualized under ultraviolet (UV) light using the ENDURO GDS Gel Documentation System (Labnet International Inc., US).

Table 3-4: Reaction setup of One Taq HotStart PCR master-mix

Reaction mix Components	**Volume/reaction (µl)**
HotStar Taq Master Mix (Qiagen)	12.5
Genomic DNA	2
Reverse Primer	1
Forward Primer	1
Distilled water	8.5
Total reaction volume	25

3.6 Acaricide resistance

3.6.1 Adult immersion test

Ticks were collected from sheep and goats randomly (September 2020), from the head, mid-section, and rear. Once ticks were collected, they were placed in plastic vials, covered with muslin cloth to allow air circulation and moisture. The collection bottleswere placed in a polystyrene box and transported to the Parasitology Laboratory at North-West University for further analysis. To assess the efficacy of commercial acaricide, the adult immersion test was conductedaccording to procedure described by Drummond et al. (1973). Upon receipt at the laboratory, ticks were rinsed with distilled water and allowed to dry on a paper towel. Ticks were then identified to species level using tick identification guide titled" Ticks of

DomesticAnimals in Africa: A Guide to Species Identification" authored by Walker et al. (2003).

Male tick species were segregated from engorged females, as only females were used for the analysis. Engorged female ticks that had already oviposited were discarded. The initial immersion solution 4% of DDE (Fluazuron 2,5% m/v, Flumethrin 1,0% m/v) was prepared in distilled water. The initial solution was then serially diluted (50%) into 10 ml of distilled water obtaining the following working concentrations (% of DDE): 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156 (Figure 3.3). Distilled water was used as the control solution. Each test experiment at different concentrations including control was carried out in triplicate. Nine groups of 2 engorged females were weighed and immersed for 30 min into 10 ml of the respective dilutions.

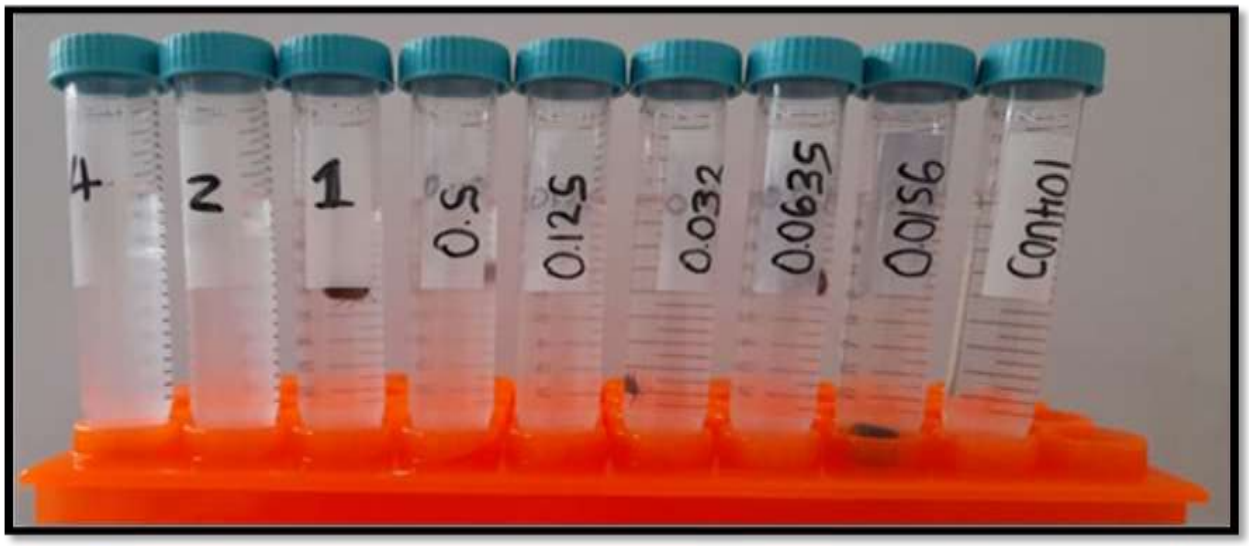

Figure 3-3: Engorged female ticks submerged in working concentrations of Drastic Deadline® and distilled water control group

Ticks were transferred onto a filter paper after being immersed in serial dilutions to remove excess solution. Ticks were transferred to glass vials covered with muslin cloth after drying and kept at 28°C in an incubator and observed for oviposition and death. As soon as oviposition was complete, the percentage of adult tick mortality and the weight of the eggs

laid by the treated ticks were recorded in comparison with the control (the ticks which did not oviposit even after treatment were considered as dead). The eggs were incubated at the same condition and the percentage of hatched eggs was estimatedvisually. The index of egg laying, and percentage inhibition of fecundity were calculated as described in literature of FAO (2004) and Goncalves et al. (2007):

1. Reproductive index (RI) = egg mass weight/live tick weight.
2. Percentage inhibition of oviposition (%IO) = [(RI control − RI treated)/RI control × 100].

3.7 Statistical analysis

Data collected was manually coded and analysed using descriptive statistics and frequencies. Microsoft® Excel 2016 and SAS Statistics (Version 9.4) statistical package was used to analyse questionnaire data. The Chi-square was used to measure the differences between the observed and expected frequencies of the outcomes of yes to no response to different variables.

Using Kochapakdee et al. (1995) formula, FECR percent was calculated. If the percentage of the FECR was less than 95% and the lower limit of the 95% confidence interval was less than 90%, then resistance was identified. Faecal egg count reduction test data were assessed using SAS Statistics for overall confidence limits (Version 9.4). Instead of using the traditional threshold values (LC 50 or LC 99), the threshold discriminating concentrations were used for EHA and LMA. The AIT data was captured on to a specially prepared data captured form and computerized for statistical analysis using SAS Statistics (Version 9.4) statistical package.

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CHAPTER FOUR

RESULTS

4.1 Questionnaire results

A total of 86 (46 and 41 small-scale farmers in DRSM and DRKK, respectively) were interviewed between December 2019 and March 2020. The demographics showed that 70% of respondents were older than 40 years of age, of which 64(74%) were males and 22 (30%) were females. Seventy percent of the interviewed farmers were pensioners, while 8 (9%) were young adults and 19 (22%) were middle aged. Most of the animals kept were for subsistence farming (60.47%) while the rest were kept for commercial reasons (39.53%). Most of the farmers in both districts practiced continuous (communal) grazing 73 (85%)while other respondents practiced rotational and seasonal grazing (Figure 4.1).

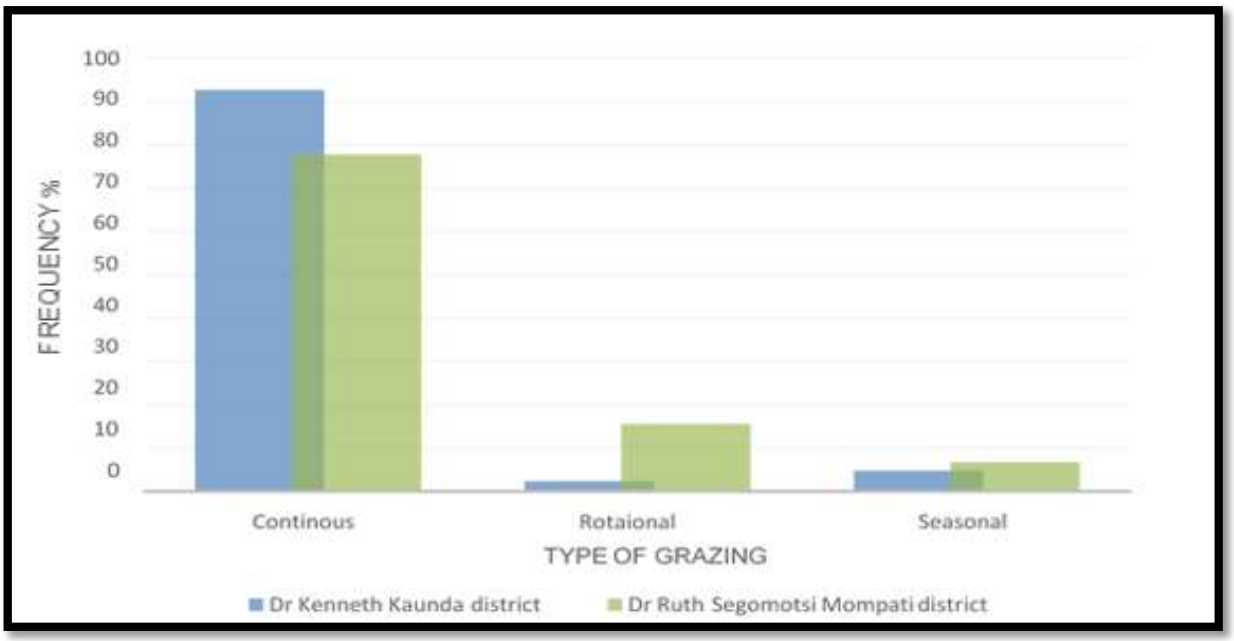

Figure 4-1: Type of grazing methods practised in DRKK and DRSM districts if North West Province.

The results of the present study revealed that 70.97% (77.28% and 65.85%) of farmers where aware of infections caused by nematodes in DRSM and DRKK respectively (Tables 4.1 and 4.2). The remaining 27.94% (21.74% and 34.15%) had no knowledge, but farmers wereable to identify common clinical signs that can be caused by (GIN) (Table 4.4).

Table 4-1: The percentages of questions relating to risk factors associated with development of anthelmintic resistance in Dr Ruth Segomotsi Mompati district

	Yes	No	Chi-square	p-value
Aware of infection	77.28	22.22	13.89	0.0002
Usage of anthelmintic drugs	100	-	-	-
Alternate anthelmintic drugs	64.44	35.56	3.755	0.0526
Knowledge of dosage calculation	84.44	15.56	24.355	<0.001
Weighing animals before drenching with anthelmintic drugs	2.22	97.78	41.0889	<.0001

Table 4-2: The percentages of questions relating to risk factors associated with development of anthelmintic resistance in Dr Kenneth Kaunda district

	Yes	No	Chi-Square	p-value
Aware of infection	65.85	34.15	4.12	0.0423
Usage of anthelmintic drugs	78.05	21.95	12.90	0.0003
Alternate anthelmintic drugs	31.71	68.29	5.480	0.0191
Knowledge of dosage calculation	85.37	14.63	20.5122	<0001
Weighing animals before drenching with anthelmintic drugs	4.88	95.12	33.3902	<.0001

In DRSM, 100% of resource-poor farmers depend on commercial drugs. Whereas 78% of farmers in DRKK use anthelmintic drugs, and the remaining farmers (11%) use indigenous worm treatment methods such as *Aloe ferox*. Respondents in DRSM alternated anthelmintic drugs more often (64.4%) depending on infection. Seventy-three (84.88%) farmers were aware of dosage requiredfor their livestock depending on the drug used. Helminth practices in both districts are presented in (Table 4.3)

Table 4-3: Farmers' helminth management activities in the districts of the North West province

	DRSM		**DRKK**	
Risk factor	Frequency	%	Frequency	%
Season of infection				
Autumn	1	2.27	4	9.76
Summer	33	73.33	26	63.41
Winter	11	24.4	1	2.44
Spring	-	-	10	24.39
Annual dosage				
Once a year	4	8.88	15	36.59
Twice a year	41	91.11	11	26.83
Thrice a year	-	-	8	19.51
Many times,	-	-	7	17.07
Common drugs used				
Benzimidazole	37	82.22	6	14.63
Levamisole	7	15.56	2	4.88
Macrocyclic	1	2.22	1	2.44
Oxytetracycline	-	-	32	78.04

Majority of farmers in both districts that used anthelmintics, declared that they treated their animals twice every year. Only 8 (19.51%) respondents reported treating animals 3times a year. Annual treatments were performed by 19 (59.33%) farmers, while only 7(17.07%) farmers dosed their animals numerous times depending on infection, particularly in sheep. Of the 86 respondents that know infection occurrence months, 58 (73.3 and 63.41%) from DRSM and DRKK mentioned that they dosed during summer, when infection rate is high.

The results of the current study showed that 30% of respondents were unaware of nematode infection and were unable to identify GIN clinical symptoms. Appetite loss (83%) and worm presence (71%) were, however, mentioned as the most common clinicalsigns of gastrointestinal infection (Table 4.4).

Table 4-4: Common clinical symptoms caused by gastrointestinal nematodes

Clinical symptoms	Frequency	Frequency (%)
Presence of worm	61	71
Emaciation	17	20
Diarrhea	59	69
Weight loss	39	45.3
Nasal discharge	12	14
Blindness	2	2.3
Loss of appetite	71	83

4.2 Faecal egg counts (FEC) and faecal egg reduction test (FECRT) results

Faecal samples tested positive for the presence of strongyle eggs. In the current study, identifiable eggs such as *Nematodirus* spp, *Trichuris* spp and *Monteria* spp. were identified

(Figure 4.2). Nematode genera including *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and/or *Teladorsagia* were determined by L3 larval identification. The egg counts in the control and treatment group are shown in (Figure 4.3 and 4.4)

Prior to treatment, the epg's in both the control and treatment groups were significantly ($p < 0.045$) different, high particularly in treatment group. Animals in the treated areas exhibited reduced epg counts compared to animals in the control areas. No significant difference ($p=0.380$) was shown in nematode egg count before treatment and after treatment.

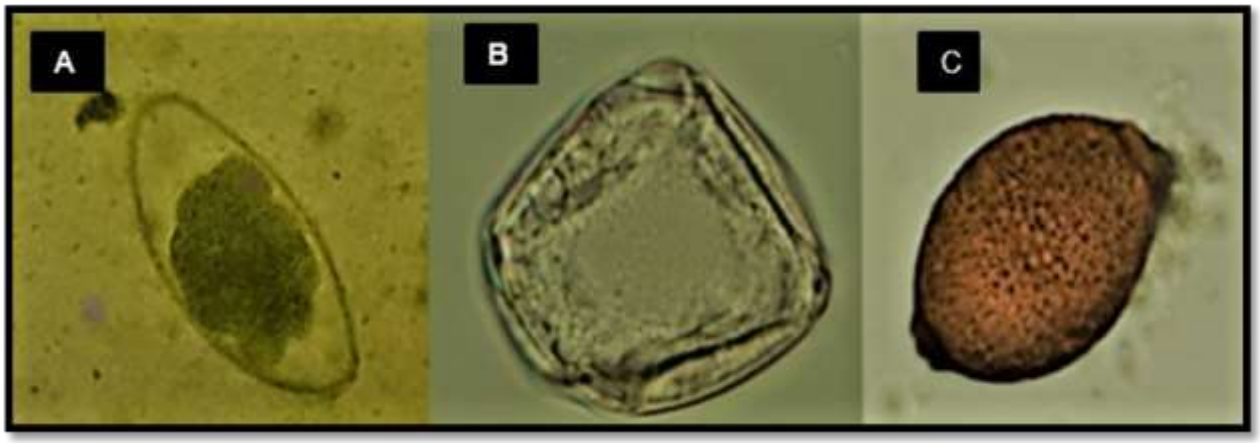

Figure 4-2: Micrograph of different parasite eggs gastro-intestinal nematode eggs observed from small ruminants from sheep and goats. A: *Nematodirus*: B: *Moniezia*, C: *Trichuris*

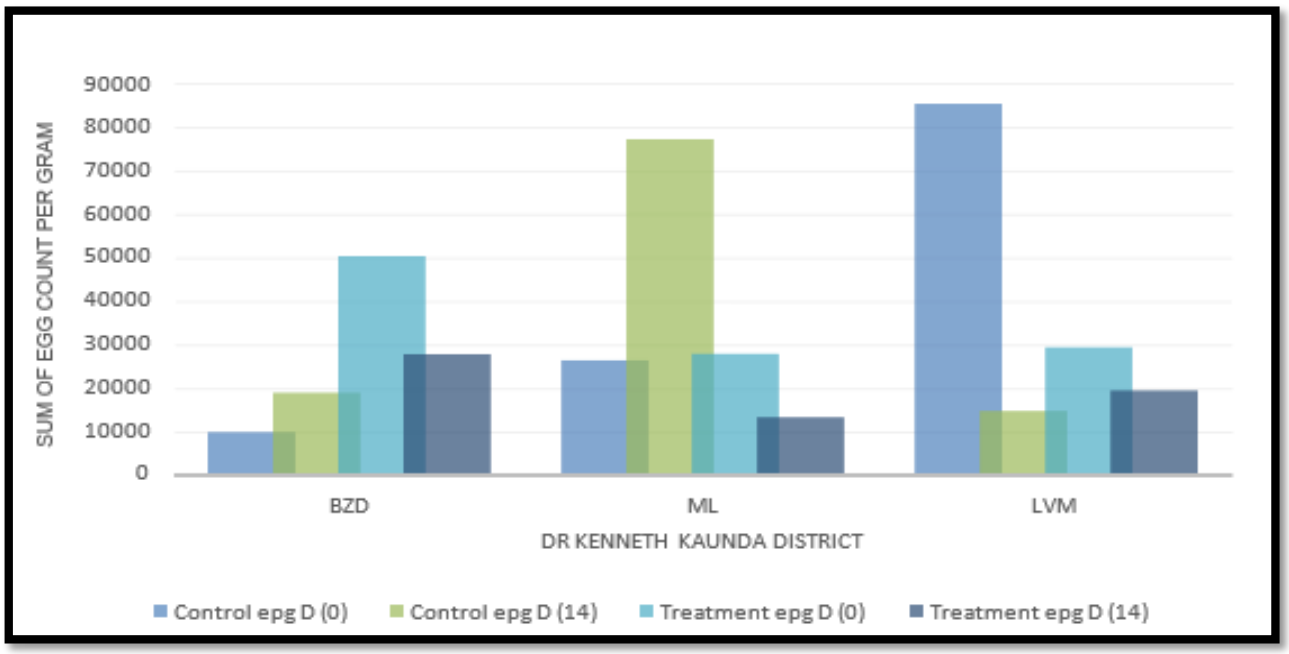

Figure 4-3: Faecal egg counts before and after in the control and treatment groups, in small-scale farmers in Dr Kenneth Kaunda districts

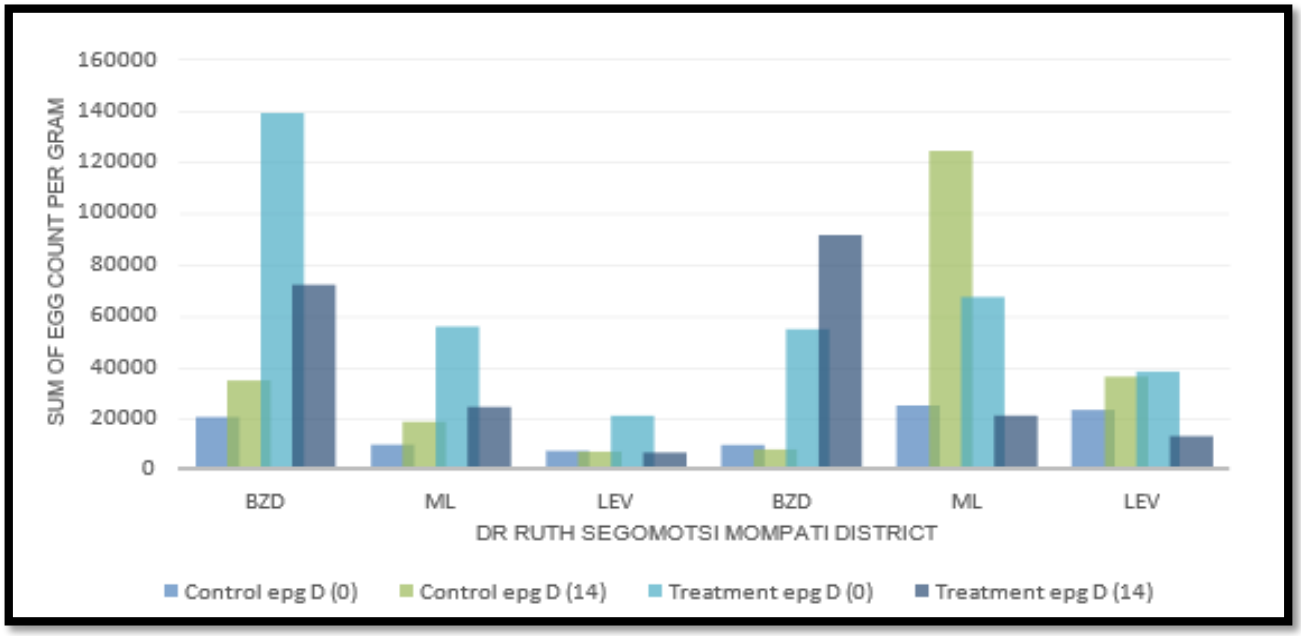

Figure 4-4: Faecal egg counts before and after in the control and treatment group, insmall-scale farmers in Dr Ruth Segomotsi Mompati districts

Faecal egg count reduction test was used to determine AR. The pre-treatment, post treatment egg counts and the per cent reduction in the faecal egg counts are given in (Table 4.7).

According to the criteria adopted, by Kochapakdee et al (1995), the results revealed development of AR against all the tested anthelmintic classes with percentages of ≤ 95% and a ≤95 lower confidence limit (LCL). This was observed in both districts of North West province. However lowest percentage of AR was detected in DRSM. An FECR% of 3.81 against albendazole, followed by 10% against LEV was detected in the same district. Resistance to IVM was tested on 3 farms, results indicated the development of AR of farm 2, 5, 8 with FECR% of 43.66, 54, and 52.81 and LCL of 17.33, 29.75 and 28.40, respectively. Treatment with anthelmintic drugs resulted in significant ($P<0.01$) FEC reduction even though, all obtained FECR% indicated resistance to major 3 classes of anthelmintic used. The coprological examination of pooled rectal samples revealed that all the animals from both districts were positive for the presence of GIN.

Table 4-5: Faecal egg count reductions and lower limits of 95% confidence levelcalculated based on individual animal's egg counts before and after treatment on the same sheep using method of Kochapakdee et al. (1995) (FECR%) = 100 x (1- [FEC2/FEC1])

District		Anthelmintic drug	FEC1 (Range)	FEC2 (Range)	FECR%	Lower limitof 95% confidence	Interpretation of results
DRSM	1	BZD	50200(1000-19300)	27700(0-11300)	3.81	-	Resistant
	2	ML	27800(1100-5400)	13300(300-2600)	43.66	17.33	Resistant
	3	LEV	32300 (1000-4800)	19400(0-300)	10	-	Resistant
DRKK	4	BZD	55455(1300-13100)	24000(100-5500)	48.28	33.42	Resistant
	5	ML	139000(1300-50300)	71600(0-34800)	54	29.75	Resistant
	6	LEV	19600(700-4300)	6200(200-12000)	66.06	54.52	Resistant
	7	BZD	109000(3400-54500)	18200(300-9100)	72.15	70.46	Resistant
	8	ML	67100(1800-13700	20600(100-4000)	52.81	28.40	Resistant
	9	LEV	37900(100-8800)	13600(0-5400)	72.95	53.98	Resistant

BZD = Benzimidazole (Valbazen®); LEV = Levamisole (Tramisol Ultra®); ML = Macrocyclic Lactones (Ivomec®); FEC1= faecal egg count pre-treatment; FEC2= faecal egg count 14 days post-treatment.

4.3 Egg hatch assay results

The anthelmintic activities with respect to egg hatch inhibition (%) of the different BZD were investigated at varying concentrations as shown in table 4.4. According to Coles et al. (1992) and Čudeková et al. (2006) at (DD) of 0.1 ug·ml-1 TBZ 99% eggs should be inhibited from hatching rendering them susceptible, however if eggs hatch, they are resistant. Thus, the percentage of hatched eggs is a direct indicator of BZ-resistant eggsin the sample.

In this study the percentage of hatched eggs ranged from 56 to 65% in 3 farms. Rate of hatching eggs was significantly higher in the control group ($p<0.01$). The percentage of eggs hatching in the negative controls (DMSO) for all flocks was >95%. The percentage of eggs hatching at the discriminating dose reveals the percentage of eggs immune to BZD in the study.

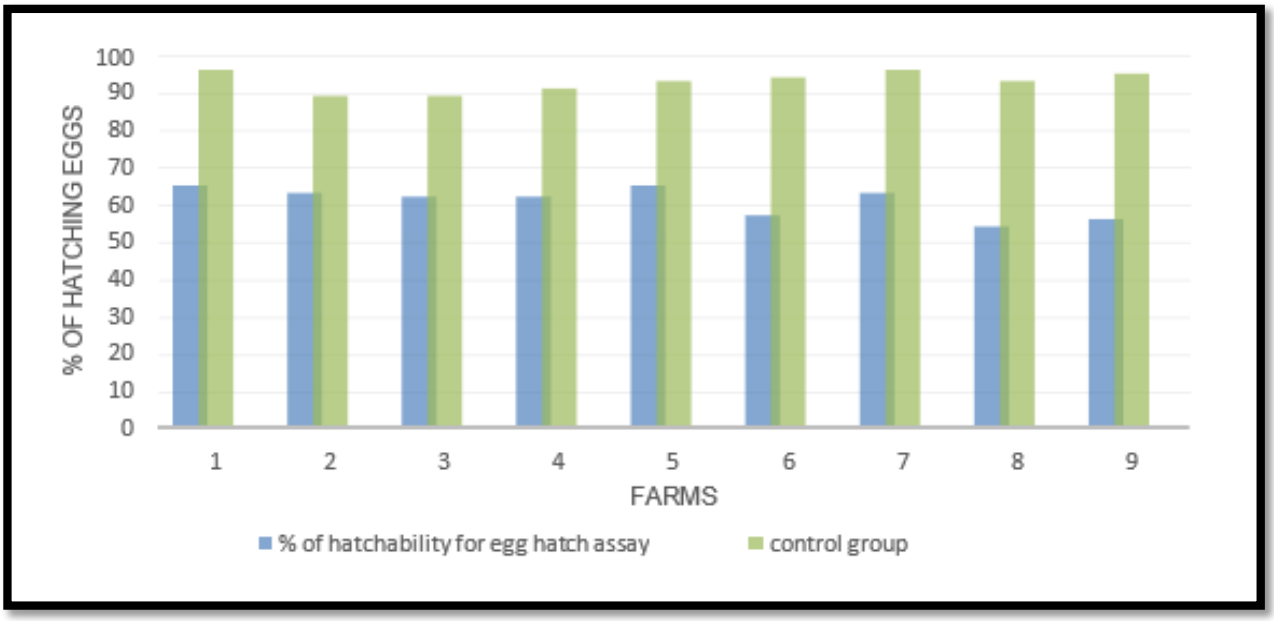

Figure 4-5: Farms with different percentage levels of hatched eggs at a threshold DD of 0.1 ug·ml-1TBZ in EHT

4.4 Larval mortality assay results

Tests were carried out to determine the effects of TBZ on the inhibition of larval mortality at various concentrations. The figures below summarize the findings (Figure 4.6). Pooled samples from different districts were investigated. The larval mortality assay using different concentrations of TBZ against time periods of 0, 2, 24 and 48 hours indicated that the rate of mortality increased with time and concentration. In this study, faecal samples per district from sheep and goat smallholder farmers were pooled representing parasites from both ruminants. However, at DD of 0.1 ug·ml-1 TBZ percentage of mortality ranged from an average of 16.01, 30 and 39 % at 0,2 and 24 hours, respectively. At DD of 0.1 µg/ml TBZ, the LMA results showed development of resistance with 50 percent of L3 surviving. The percentage of mortality was higher for treated plates (P<0.01) than for untreated control, although resistance was detected

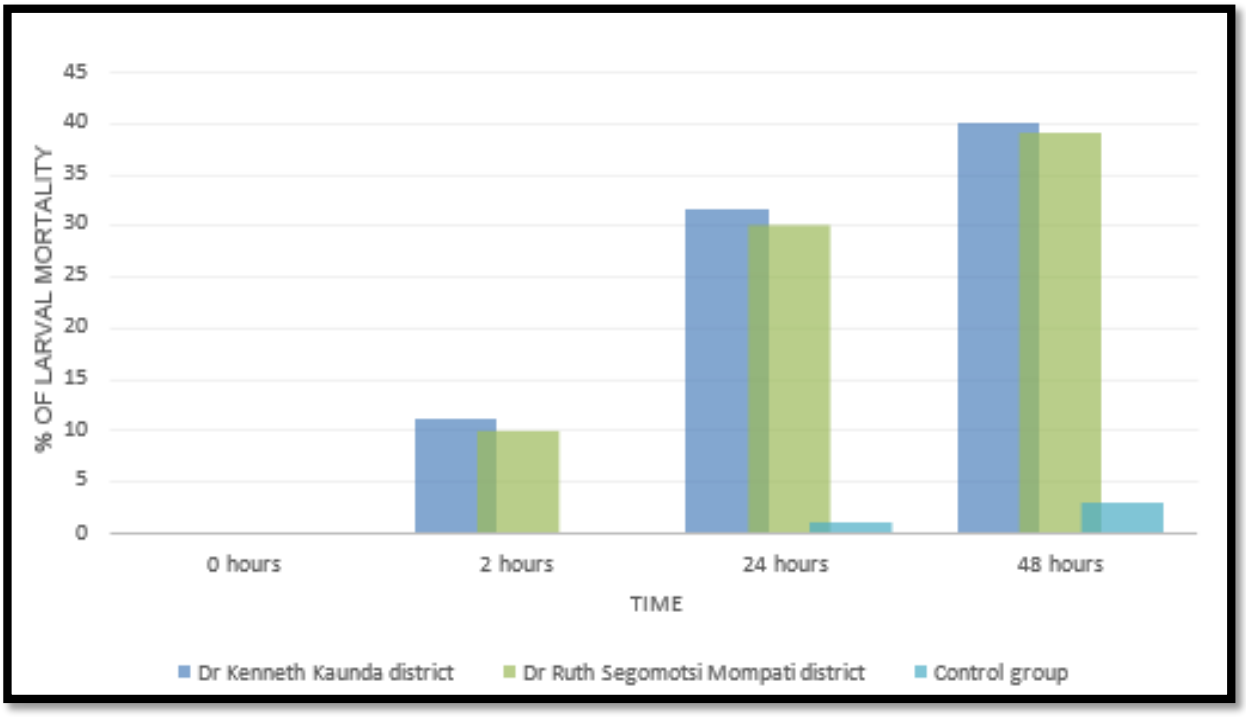

Figure 4-6: The percentage of larval mortality in third larval stage (infective) in discriminating dose of TBZ (0.01 µg/ml) in the larval mortality assay

4.5 Detection of resistant nematodes using PCR assay

A total of 83 faecal samples from both sampled districts were positive for the presence of nematode eggs by microscopy. The DNA was extracted from pooled faecal samples after treatment, per farm. Faecal samples were screened for the presence of *Haemonchus*, *Oesophagostomum, Trichostrongylus* and *Teladorsagia.* Amplification of ITS2 gene by PCR showed bands at 256 bp and 333 bp (Figure 4.7 and 4.8). This is positive results for *Haemonchus* on farm 1, 2, 4, 6: ITS-2 gene amplicons (256 bp). Resistant in all the three drug classes tested except *Haemonchus* spp. was not detectedby PCR at farm 6 and 8, which as treated with LEV and ML respectively. *Oesophagostomum* spp was detected in farm 1, 2: ITS-2 gene amplicons (333 bp)., while farm 2, 4, 5 and 6 tested negative for *Oesophagostomum* spp, which were treatedwith ML and LEV. Nematode *Trichostrongylus* and *Teladorsagia* tested negative in all the farms in both districts.

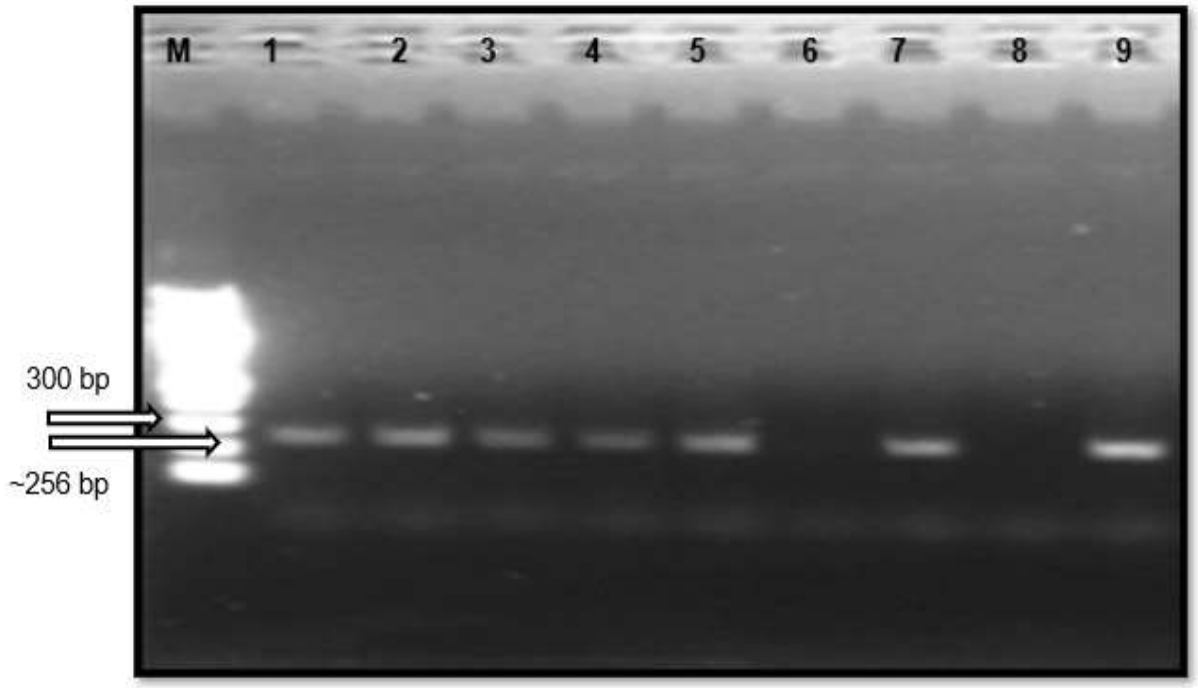

Figure 4-7: PCR positive for the presence of *Haemonchus* spp

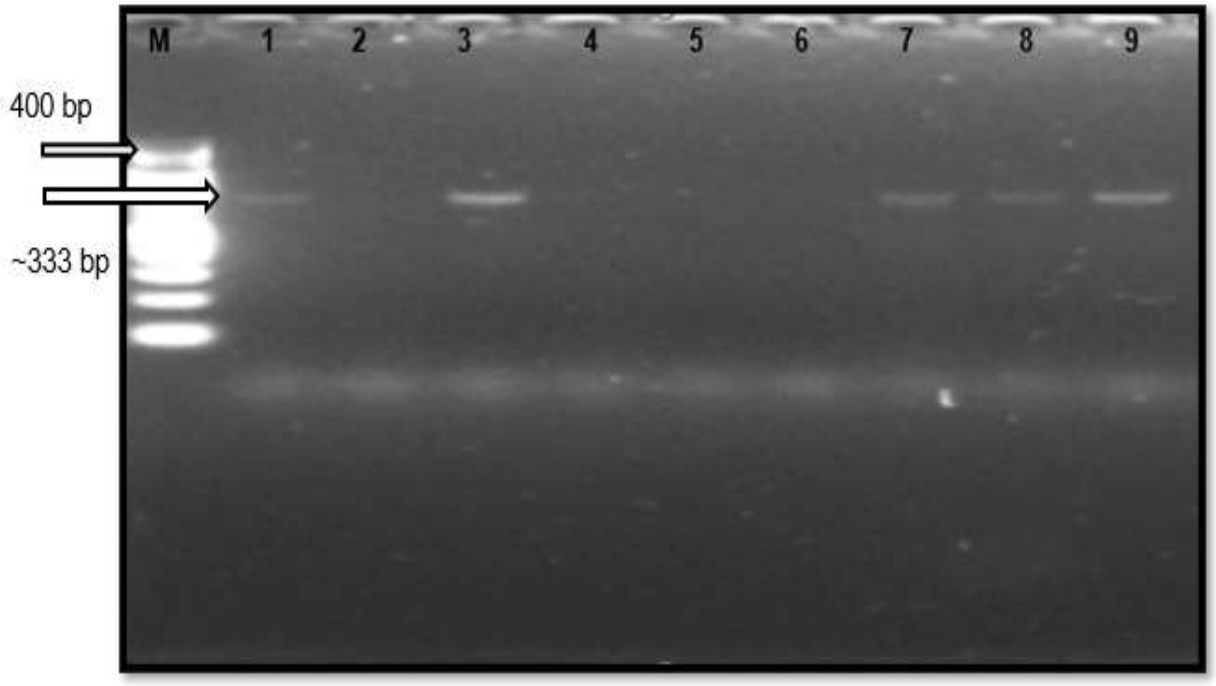

Figure 4-8: PCR positive for the presence of *Oesophagostomum* spp.

4.6 Adult immersion test results

The AIT was used in this present study to determine the relative effectiveness DDE against ticks in small ruminants. In total 20 ticks were collected, and all the species were identified. The most abundant tick collected from both sheep and goats was *Rhipicephalus* species. A taxonomy identification confirmed *R. evertsi evertsi* as photographed under a stereomicroscope as shown in (figure 4.9). Male *R. evertsi evertsi*was characterized by small red convex eyes on the dorsal side, with distinct caudal appendages on the ventral side. While female ticks were identified based on dark browncolour scutum and orange to red legs.

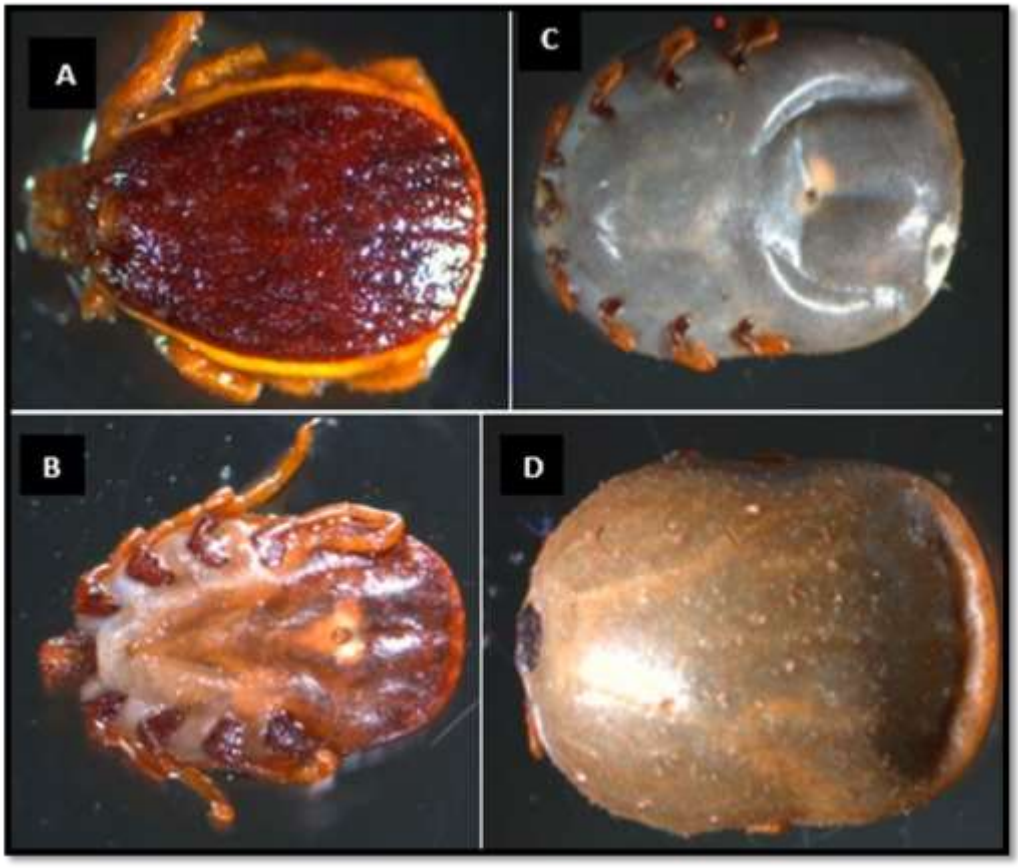

Figure 4-9: Micrograph of morphological identification of *Rhipicephalus* species. A: Male dorsal view (red convex eyes). B: ventral view (caudal appendages which are absent in females). C: engorged female ventral view. D: dorsal view (dark brown colour scutum)

The percentage of adult mortality, reproductive index (RI) and tick oviposition inhibition (IO) were found to be significantly inhibited in a dose-dependent manner.

In the AIT, effects of treatments on engorged females were assessed by measuring egg hatching rate, mortality, and oviposition rate. In this study DDE was toxic to the engorged adult female ticks resulting in 100% mortality, even though mortalities were found to be significantly inhibited in a dose-dependent manner. Ticks exposed to lower concentrations died after 3-4 day while ticks exposed to higher concentrations died after a 1-2 days. However, in the control group, mortality was observed after oviposition. Ticks were monitored daily for 7 days, all females exposed to DDE at all varying concentration did not go through oviposition (Table 4.6). At concentrations (0, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0321 and 0.0156 µg/mL), efficacy was observed against engorged females, rendering them susceptible to DDE

Table 4-6: Acaricidal effect of Drastic Deadline extreme® (fluazuron 2.5% and flumethrin 1%) against engorged females of *Rhipicephalus evertsi evertsi*

	Engorged female ticks	Egg laying		%	%
Active ingredient	**FMW (G)**	**EG (g)**	**RI**	**IO%**	**Efficacy**
Treatment group (fluazuron 2.5% and flumethrin 1%)	0.22(0.01-0.56)	0	-	0	100
Control group (distilled water)	0.14 (0.3-0.0.14)	0.01-0.07	0.01-0.47	-	-

FMW= female mean weight; EG=egg weight; Reproductive index (RI) = egg mass weight/live tick weight; Percentage inhibition of oviposition (%IO) = [(RI control − RI treated)/RI control × 100].

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In the present study, the knowledge, and perceptions of resource-poor small ruminant farmers in North West province of South Africa was assessed. Demographics revealed that most of the farmers were males compared to female farmers. Education level had a significant association with farmers' awareness about AR, as well as their knowledge, attitudes, and practices toward sustainable worm control. The study participants relied on both the government's social grants and livestock farming as a source of income.These findings are comparable to those of Luseba and Van der Merwe (2006) and Yawaet al. (2020), who articulated that majority of rural farming communities depend on livestock farming as a secondary form of income apart from social grants. Subsistence farming was the common form of farming, relying on natural resources as food supplement for their livestock. This practice of farming is associated with relatively poor-quality feed and often in marginal environments (Materechera 2010), exposing livestock to variety of parasite infestation which in turn affects their wellbeing (Muchadeyi et al. 2007).

As a result, 77.28% and 65.85% of farmers in DRSM and DRKK respectively, indicated that they were knowledgeable about parasites and reported that relative to external parasites (ticks), GIN were most problematic. Similar report was also provided by Mwale and Masika (2009) and (Njunga 2003). In another South African study by Tsotetsi et al. (2013), 88% of farmers were aware of GIN, which is much greater than the results observed in this present study but not less than that recorded by 57% of farmers in Limpopo according to a study by Mphahlele (2020). Most of the farmers (89%) in the study treated their animals with anthelmintics. Commonly used commercial drug classes included BZD (90%), OXY (78.04%), LVM (18%) and ML (3.44%). According to the farmers BZD class group is effective and affordable compared to other anthelmintics, thus it was frequently preferred for control of GIN

(Demeler et al. 2009). However, this explains the rapid development of resistance against this class. The injectable OXY was considered as an anthelmintic and highly effective against helminthosis. Most farmers in DRKK (78%) considered it to be a medication that can cure any form of parasite-caused disease. Similarly, studies by Getchall et al. (2002) and Sekyere (2014), in Mafikeng and Ghana respectively, found that most farmers used OXY as a common drug for a variety of ailments affecting their livestock. There seemed to be a misunderstanding reflecting a lack of awareness about anthelmintics and antibiotics. Subsequently, to reduce activities that would increase antibiotic misuse and susceptibility, increased veterinary focus and education should be rendered to small scale farmers.

An overall total of ninety-six farmers in DRSM (97.78%) and DRKK (95.12%) mentioned that they used commercial drugs. However, 83 (96.51%) respondents used visual appraisal to determine live animal weight. Only 4% of farmers in both districts weighed their animals before dosing, consequently, leading to cumulative possibility of under/overdosing through incorrect weight estimation. These findings are also in accordance with observations reported by Mukaratirwa et al. (1997); Vatta et al. (2001); Bakunzi (2003); Tsotetsi et al. (2013); Mphahlele et al. (2019); Ramabu et al. (2020). Mphahlele et al. (2019) have emphasized that to ensure the correct dosage, livestock farmers must assess the weight as precisely as possible, particularly by measuring each animal individually. However, this is an ongoing concern due to the lack of adequate farming resources and government services (Nixon et al. 2020).

Frequency of anthelmintic treatments is another factor in the emergence of resistance in small ruminants raised in communal farms. The results of this study showed that most farmers in both districts drench their livestock twice a year (58.97%), followed by once a year (22.75%) and three times a year (19.51%) respectively. A study conducted by van Wyk et al., (1999) in Limpopo revealed that less intensive treatment is among the issues that contributed to development of AR, which agrees with the present study. However, the above results contradict the observations reported by Sargison (2011) study, which supported the notion that intensive frequent annual treatments, contribute immensely to

unprecedented development of AR, which is consistent with the following studies (Shalaby 2013; Geary et al. 2015; Atan and asio-Nhacumbe et al. 2017; Stewart et al. 2020). To solve this issue, according to Vijayasarathi et al. (2016), it is mandatory to balance less/ highly intensive frequencies to avoid the onset of resistance. Target deworming should be considered to avoid under/overdosing against selection of resistance to commonly used drugs.

Farmers indicated that in summer and spring, animals were often given doses. Higher GIN prevalence has been observed during these seasons where optimal climatic conditions are favorable for GIN survival, development, and distribution (Yadav et al. 2006). Based on their responses in this study, 30% of respondents were unaware of nematode infections, however in contrary all 100% (86) farmers identified clinical symptoms caused by GIN. Common symptoms included loss of weight 83% followed bypresence of worms 71%. According to Vatta and Lindberg (2006) there seems to be a greater awareness about tapeworms than of the more dangerous roundworms. This is probably because the tapeworm proglottids are easily visible with the naked eye on the dung of the animal. It should, however, be considered that different GIN infections display common signs that can result in misdiagnosis, which in turn affect anthelmintic efficacy.

Questionnaire survey results in this study indicated that 89% of farmers use the same drugs for treatment, resulting in lack of rotation of anthelmintics, and this is regarded as a contributing factor to the development of AR (Martin et al. 1989; Martinez-Valladares etal. 2013). According to Shalaby (2013), drenching with two anthelmintics from different drug classes is one preventive method during the early stages of AR development. In this study, farmers were knowledgeable about the dosage, however relied on visual appraise to determine weight of animal. Bakunzi (2003) research study on AR of nematodes in South African communally grazed goats, identified underdosing as a risk factor for the progression of AR. To determine the necessary anthelmintic dose, the findings concluded that AR is elevated in communal farms due to underdosage caused by visual measurement of animal weight as opposed to weighing livestock and dosing according to manufacturer. In majority of cases treatment frequencies were influenced by clinical conditions of animals,

which results in selection pressure in which resistant nematodes dominate in the host and refugia.

The coproculture analysis revealed that in both districts common distinguishable nematodes included *Moniezia* spp., *Nematodirus* spp., *Trichuris* spp. and strongyle eggs. Presence of AR development was detected by *in vivo* and *in vitro*. The results of the FECRTs in both districts against all 3 broad-spectrum anthelmintics indicated development of resistance. Highest level of AR was recorded on one farm (3.81%) against BZD in the current study, and this is consistent with the findings by Praslička et al. (1994); Čerňanská et al. (2006), who reported 10% AR development in Slovak Republic which is alarming to still see how efficacy is deteriorating far more. Gastrointestinal nematodes present in both sheep and goat populations were found to bemore resistant to BZD and LEV as compared to ML. Research finding by Bakunzi (2008)also reported 68% and 58% efficacy in the BZD and LEV, respectively. Mphahlele (2020), recently recorded a similar pattern of results, with resistance of 6.7%, although the results obtained in this analysis are lower. Earlier studies conducted by van Wyk et al. (1999), reported resistance against BZD, LEV, and ML in 90% of farms tested. A variation of factors contributes to the outcomes of these results. According to Bosco et al. (2020) BZD is regarded as the leading anthelmintic class and is considered the most used by farmersdue to its efficacy, affordability, ability to be used for pregnant animals. The AR problem has been reported in many parts of the world Ethiopia (Eguale 2009), South America (Canevari et al. 2014), Malawi (Leahy et al. 2017); Sudan (Mohammedsalih et al. 2019).Majority of the studies reported resistance against BZD. Resistance against LEV rangedfrom 10% to 72.95%. A similar conclusion was reached by Cezar et al. (2010). Macrocyclic lactones (3.44%) was third commonly used anthelmintic class and AR development from this class was also detected in this study with FECRT% ranging from 43% to 58%. Resistance to ML is less common, according to Papadopoulos et al. (2012).However, systematic review and meta-analysis study by Baiak et al. (2019) observed thatthere were 95% of the articles reporting resistance against ML while 1% of the studies reported efficacy from studies conducted between 1992 and 2012.

To further validate obtained FECRT% observations, EHA was conducted for the detectionof BZD resistance in GIN of small ruminants. Egg hatch assay was conducted as described by Coles et al. (2006). indicating that at DD of 0.1 ug·ml-1 TBZ 99% eggs should be inhibited from hatching rendering them susceptible, however if eggs hatch, theyare resistant. Results obtained in this study identified the presence of AR on all the farms (100%). Hatching percentage on farms in both districts ranged from 56% to 65%%. These results indicate the presence of resistant parasites on experimental farms and is consistent with findings of past studies by Čudeková et al. (2010), which reported a resistance of 80% to 90% in *Haemochus* spp. isolates in small ruminants. According Molefe 2012, ability of eggs to hatch could also depend on species diversity, developmental stages of the parasite and the type of environmental conditions they thrive in. However, one limitation of this technique is that AR was detected against one drug class only. New BZD classes such as fenbendazole, and other anthelmintics such as LEV and ML are considered unstable due to poor solubility, hence less ovicidal effect. Not all anthelmintic drugs can prevent GIN from embryonation and hatching. Relating EHA results with FECRT showed a correspondence in terms of development of resistance. Farmers in the North West province that relied on BZD to treat their livestock were 14.63% in DRKK and 82.22% in DRSM. As a result, a low FECR of 3.81% was observed in DRSM against BZD, corresponding to the high number of hatching eggs (65%). Secondly EHA resistance could be a result of lack of rotation. Sixty-five farmers in DRSM admitted to not changing brands, which is also a contribution to detection of resistance inconducted assays. The ovicidal effect of BZD was also previously recorded by Mphahlele(2020), resulting in very low hatching rates of 5% to 30% percent, while FECRT% ranged from 47% to 96%, which is marginally higher, compared to the current study. From obtained results, correlation between EHA and FECRT% completely agreed on development of resistance in all farms tested.

Monitoring of susceptibility/resistance of anthelmintic drugs in different strongylid nematodes is important considering the widespread development of AR. The results of LMA clearly showed that AR was present in all the experimental farms. The LMA results showed resistance with 50% of surviving L3 at DD of 0.1 µg/ml TBZ. Polymerase chain

reaction confirmed the presence of *Haemonchus* and *Oesophagostomum* spp. in sheep and goats belonging to small scale farmers in 2 districts of North West province. Surviving genus *Haemonchus* was detected in the farms 1,2,3,4,5,7,9, and was the most predominant nematode after treatment against all 3 drug classes used. Followed by *Oesophagostomum* which was the second most predominant at farms 1,3,7,8,9, predominately against BZD and LEV. These findings are consistent with other studies conducted in small-scale communal farming systems in South Africa (Van Wyk et al. 1999, Bakunzi 2003, Montshwe 2006, Tsotetsi et al. 2013, Siyabulela et al. 2020).

According to Varady et al. (2011), compared to other nematodes that affect small ruminants, *H. contortus, O. circumcincta* and *T. colubriformis* seem to be a highly sensitive and resistant population. Several studies indicate, however, that there is discrepancy between *in vivo* and *in vitro* experiments (McIntyre et al. 2018). A study by Crook et al. (2016) reported resistance against BZD in EHA and LMT, however FECRT indicated susceptibility against the same drug. This is probably because under field conditions FECRT sensitivity decreases, and another contributing factor is species diversity, which have a direct effect on diagnosis. Outcomes of all the tests conducted inthe current study indicate the development of AR in all flocks.

Although resistance was not detected in the present study using DDE, extensive research on ACR has been reported against carbamates and organophosphates and synthetic pyrethroid has been extensively reported Mekonnen et al. (2002), Tønnesen et al. (2004), Horak et al. (2018), indicating need for sustainable approaches to attain efficacy. According to Yawa et al. (2020), factors such as the absence of ACR test, illegal selling of acaricides, and the absence of training for farmers on the use acaricide, are drawbacks associated with communal farming in rural communities. In the present study farmers prefer to purchase commercial drugs with dual purpose, that have ability to eliminate both tick and helminth simultaneously instead of targeted drugs.

5.2 Conclusion

In conclusion, the findings of this study demonstrate that, though majority of the farmer participants in the two districts of North West province had adequate knowledge about helminthosis, they all practiced poor parasite management and AR mitigation practices. This discrepancy between knowledge and practice raises various challenges and opportunities, for example surrounding the use of the same anthelmintics, monitoring of parasitic infections, frequency of drenching and weighing of animals. Despite GIN infection was regarded more problematic as compared to ticks, the hypothesis of this study was successfully proven that management and treatment strategies have impact on development of resistance on strategy on sheep and goat farms in North West province. The occurrence of different levels of resistance against BZD, LEV and ML in GIN, as evidenced by the FECRT was consistent with previous studies conducted in communal farms in South Africa. High levels of resistance were detected against *Haemonchus* and *Oesophagostomum* spp. The study failed to detect resistant strains of either *Trichostrongylus* spp and *Teladorsagia* spp. Elsewhere, resistant strains of these genera have been reported in small ruminants (Conder and Campbell 1990; Mukaratirwa et al. 1997). It is possible that resistant populations of the two genera were omitted in these flocks because they were absent or simply present in limited populations at the time of sampling. Resistance to ticks using AIT was not detected, contradicting the study's hypothesis. However, the study proved practically resistance against commonly used

anthelmintic drugs, suggesting the need to redress the worm control programme for small ruminant farmers in North West province.

5.3 Recommendations

To reduce the costs caused by AR and ACR, treatment campaigns in communally raised small stock should be implemented. Inability to weigh livestock seem to be the most common mistake in communal farms. Instead of using electric weighing scales/ mechanical spring scale, farmers can use heart girth measurement tape to determine liveweight, which can be used for all types of ruminants. Low frequency of drug treatment with the same anthelmintic drug increases the selection pressure. While resistance to acaricides was not identified in this research project, to avoid repeated and excessive treatment resulting in resistance, reliance on commercial drugs can be coupled with traditional anthelmintics for control measure. The *in vivo* and *in vitro* assays regarding risk of AR and ACR assessments need to be implemented as routine tests in provinces where small-scale farming is commonly practiced giving a broader picture of variety of parasites affecting livestock and realising common mistakes that contribute to development of resistance.

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ANNEXURE ONE: QUESTIONNAIRE

	Questionnaire no	

Name of interviewer **Leina la mobotsitherisano**	
Date of interview **Letsatsi la dipotsotherisano**	

Demographic Information

Name and Surname: **Leina le Sefane**				
Physical Address: **Aterese ya legae**				
Cell phone number: **Nomoro ya mogala wa letheka**				
Village **Motse**				
Ward no. **Nomoro ya wade**				
District **Setereke**				
Municipality **Masepala**				
Locality co-ordinates **Sebaka le dikhoodineiti**				
Gender **Bong**	Male **Bonna**		Female **Botshegadi**	
Age group **Sethopha sa dingwaga (Bogolo)**	Youth **Mosha**	Middle age **Dingwaga tsa bogareng**		Pensioner **Phenshene**
Education Level **Thutego**	Primary and below **Poraemari**	High school **Sekolong se segolo**	Post matric qualification **Materiki**	University degree **Dikirii ya yunibesithi**

1. Which other animal species do you keep, and how many are they?

Species **Mofuta wa phologolo**	**Number of animals** **Palo ya diphologolo**
Cattle/**dikgomo**	
Donkeys/**ditonki**	
Goats/**dipudi**	
Sheep/ **dinku**	
Poultry/**dikgogo/dikoko**	
Pigs/**dikolobe**	
Total	

2. Why do you keep these animal species? **Ke ka ntlha yang o nale leruo?**

Personal **Ka mabaka a botho**		Business **Ka ntlha ya kgwebo**		Other **Ka ntlha tse dingwe**	

If other, please specify. **Ga karabo e le ka ntlha tse dingwe, tlhalosa.**
……
……

3. Where do you purchase your farmed animals? **O reka kae diwuiwa tsa hao?**
……
……
4. How long have you been keeping livestock? **O na le dingwaga di le kae o rua?**

Less than 5 years **Ka fa tlase ga dingwaga di le tlhano (5)**		From 6 to 10 years **Go tloga dingwaga di le thatar go ya di le lesome**		More than 10 years **Go feta dingwaga di le lesome**	

5. What type of grazing method do you use for your animals?

Continuous grazing		Rotational grazing		Seasonal grazing	

6. What type of feed do the animals eat? **Ke mofuta ofe wa dijo o fepang diphologolo tsa hao**

Plant material **Dimela**		Hay **Bojang**		Straw **Letlhaka**		Lucerne **Luserene**	

7.Are you aware that animal can have worm infection? **A o itse ka diboko tse di tshwaetsang leruo?**

Yes/**Ee**	
No/**Nyaa**	

7.1 If yes, indicate clinical signs for worm infection. **Ga karabo e le ee, tlhalosa matshwao a**

bolwetse ba diboko

……
……
……

8. How do you control worm infections in your livestock? **O laola yang dibokwana tse di tshwaetsang leruo?**

……
……

9. Do you treat your animals with commercial drugs? **Ebang o phekola diphoofolo tsa hao ka meriana ya sekgowa**

Yes **/Ee**	
No/**Nyaa**	

9.1 If yes, please specify. **Haeba karabo e le Eya, bolela meriana eo o e sebedisang**

……
……

10. At what season do your animals show the signs of worm infection? **A o itse dikgwedi tsediphologolo di tshabellwang ke bolwetse jwa diboko?**

Summer **Ka selemo**		Winter **Ka mariga**		Autumn **Ka letlhabula**		Spring **Ka dikgakologo**	

11. Do you ever change the brand name that you use? If yes how often and why? **A o na le go fetola melemo/ditlhare tse o di dirisang go alafa diphologo? Ga karabo e le ee, o e fetola ga kae? Goreng o e fetola?**

Yes/**Ee**	
No/**Nnyaa**	

12. Do you know how much medicine you must give to your animals**? A o itse selekano/selekanyo sa melemo/ditlhare se o tshwanetse go se fa diphologolo?**

Yes/**Ee**	
No/**Nnyaa**	

13. Do you weigh your animals before dosing? If yes, how, and why? **A o lebella boima/bokete jwa diphologolo tsa gago pele o di fa melemo/ditlhare? Ebang o bega boima ba diphologolo pele o di fa/nwesa meriana.**

Yes/**Ee**	
No/**Nnyaa**	

……
……
……

14. Do you know if the remedies are working/effective? If yes how do you know? **A o a itse goremelemo/ditlhare e a fodisa? Ga karabo e le ee, o itse jang?**

Yes/**Ee**	
No/**Nnyaa**	

……
……

15. Where do you find your pest management information with regards to worm treatment? **Lintlha tsa hau tsa tsamaiso ea likokonyana li u li fumana kae mabapi le kalafo ea seboko**

Animal health technician **Motegeniki wa pholo ya diphologolo**		Sales representative **Moemeli oa thekiso**		Other farmers **Balemirui ba bangwe**		Other **ntlha tse dingwe**	

If other, specify. **Ga karabo e le ka ntlha tse dingwe, tlhalosa.**

……
……

16. How often do you administer medicine to your livestock **O alafa leruo la gago hakae?**

Once a year **Gangwe fela mo ngwageng**		Twice a year **Gabedi ka ngwaga**		Thrice a year **Kararo ka ngwaga**		Many times **Makgetlo a le mantsi**	

17. Do you ever use alternative medicine to treat your animals against internal parasite infestation? **A o dirisa mefuta e mengwe ya kalafi go alafa bolwetse ba diboko?**

Yes **Ee**	
No **Nnyaa**	

If yes, what do you use. **Ga karabo e ee, tlhalosa dikalafi/ melemo o e dirisang**

……
……

18. Any other comments on the usage of anthelmintics to treat worm infection in livestock? **Tshedimosetse efe kappa efeng ya meriana eo o e sebedisang ho phekola tshwaetso tsa mayoha diphofolong tsa gago? A o na le tshwaelo ka tiriso ya melemo/ditlhare ya/tsa diboko go alafa leruo?**