

**EFFICACIES OF CHLORFENVINPHOS, AMITRAZ AND
ALPHACYPERMETHRIN AND THEIR COMBINATIONS ON
RHIPICEPHALUS (BOOPHILUS) DECOLORATUS TICKS FROM CATTLE
IN NANDI COUNTY, KENYA**

**BY
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DECLARATION

DECLARATION BY CANDIDATE

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DEDICATION

To all who contributed towards this research work.

ABSTRACT

The one-host tick, *Rhipicephalus (Boophilus) decoloratus*, is a ubiquitous ectoparasite of cattle in Nandi County, Kenya. For more than one decade, the County has used plunge dips to apply, either chlorfenvinphos, amitraz and alphacypermethrin acaricides on the cattle, to control the tick. Farmers, reportedly combine different classes of acaricides for use too. This study was designed to examine the dipping performance in the years 2000 – 2012, assess the efficacies of the acaricides and their binary combinations on the adult female *R. (B). decoloratus* ticks and larvae. Visibly engorged ticks that persisted dipping with the acaricides were collected from cattle and subjected to the Adult Immersion Test (AIT) using the acaricides, at recommended dipping concentrations. Larvae from the ticks were tested with similar acaricide preparations through the Larval Packet Test (LPT). The ticks were also tested with binary combinations of the acaricides, mixed at 50:50mls. The study showed that the majority of the farmers in the County used an average of 286 communally-managed plunge dips (84.11%) while others used 48 private dips (15.89%). Irregular numbers of cattle were presented for dipping annually (266,178.3±30,963), with anaplasmosis being the most prevalent tick-borne disease (47.74%), followed closely by East Coast Fever (47.08%). Over half of the dip-wash used in the period (56.36%) had the recommended acaricide concentrations, while 38.81% were under-strength and 4.83% over-strength. Viable ticks, whose weights ranged between 0.114 – 0.124g, with capacity to lay eggs that had visually 100% hatchability, were recovered from the cattle. The single acaricides had slow action on adult ticks, with 66.67 – 91.67% mortalities occurring after 96 hours from immersion, while combinations containing chlorfenvinphos had 83.33% and 100% efficacies on those collected from cattle dipped in alphacypermethrin and amitraz, respectively, at the same time interval. The single acaricides produced 75 - 100% efficacies on larvae in LPT, while combinations had 95 -100% in 24 hours ($F = 0.000$). In conclusion, irregular numbers of cattle were dipped with varying concentrations of acaricides, mostly in communal dips, in the County. The acaricides used were still efficacious, especially on larval stages of *R. (B). decoloratus*, with their combinations having improved efficacies against the adult and larval stages of the tick.

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LIST OF ABBREVIATIONS

ANOVA	– Analysis of Variance
DVS	– Director of Veterinary Services
EAC	– East Africa Commission
ERA	– Economic Review of Agriculture
FAO	– Food and Agriculture Organization
GDP	– Gross Domestic Product
GoK	– Government of Kenya
IGAD	– Inter Governmental Authority on Development
ILRI	– International Livestock Research Institute
KDB	– Kenya Dairy Board
KNBS	– Kenya National Bureau of Statistics
KShs	– Kenya Shillings
LPI	– Livestock Policy Initiative
MoLD	– Ministry of Livestock Development
NEPAD	- New Partnerships for African Development
PPLPI	– Pro-poor Livestock Policy Initiative
SDP	– Smallholder Dairy Project
VSD	– Veterinary Services Department
WARRC	– World Acaricide Resistance Referral Center

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CHAPTER ONE

INTRODUCTION

1.1: BACKGROUND INFORMATION

Livestock contribute both directly and indirectly to human livelihoods through food production, supply of industrial raw materials, environmental conservation and job creation amongst others (Alexandratos and Bruinsma, 2012; Upton, 2004).

Globally, livestock are an asset worth \$1.4 trillion and its market chains employ at least 1.3 billion people and directly support the livelihoods of estimated 600 million smallholder farmers in the developing world (Lanyasunya *et al.*, 2006; Thornton, 2010). The enterprise is also an important risk-reduction strategy for resource-poor communities and as a source of food, labour and manure for crops in the smallholder farming systems (Scott, 2006; Rosegrant *et al.*, 2009). The livestock sector is among the effective agricultural activities that promote poverty reduction programmes in agriculture-based economies, when compared to GDP growth in other sectors (World Bank, 2007). Rapid population growth, urbanization and improved incomes, as a result of industrialization in developing countries, have made the sector to be one of the fast-growing agricultural subsectors, due to increased demand for livestock products (Delgado, 2005).

Smallholder-based agriculture is an important economic sector in East Africa. Studies by Salami *et al.* (2010) found that the smallholder farmers in the region accounted for 75% of the entire agricultural production and over 75% employment. Out of the contribution, cattle and poultry dominated the livestock sub-sector.

Livestock farming in Kenya is a source of livelihoods that is associated with much socio-cultural attachment (Onono *et al.*, 2013). The dairy industry is reputed as the

most advanced part in the livestock sub-sector in the country's agricultural sector (Muriuki *et al.*, 2004), and the largest dairy cattle herds in the sub-Saharan Africa (Karanja, 2003). Most of the cattle are found in smallholder farms (Omore *et al.*, 1999).

The 2009 census established that Kenya had a cattle population consisting of 3,355,407 exotic dairy breeds and 14,112,367 indigenous breeds (Kenya Population and Housing Census, 2009). This population was reputed to constitute the single largest subsector of agriculture in Kenya (IFAD, 2006).

However, the cattle face numerous challenges from ticks and tick-borne diseases among other constraints (Gitau *et al.*, 1997; Wesonga, 2010). Losses from tick infestation include reduced growth rates, blood loss and transmission of a wide range of pathogens (Jongejan and Uilenberg, 2004). The tick-bites irritate cattle, thereby interfering with their feeding, and also produce wounds that predispose the livestock to secondary infections, and reduce the quality of hides or skins produced by the infested animals (Wesonga *et al.*, 2010; Taylor and Wall, 2016). Omore *et al.*, (1999) observed that more ticks and tick-borne diseases were found on livestock raised in open grazing fields than on the confined intensive production systems as zero-grazing units.

The one-host tick *Rhipicephalus (Boophilus) decoloratus* (Koch, 1844), in particular is a common parasite of cattle, sheep, goats and donkeys, including wildlife, in Eastern Africa (Walker, 2003). It is active throughout the year and prefers to attach on the shoulders, neck and dewlap of the host. They are also found in upper edges of the ears and on legs. The tick requires moisture and warmth in the environment for propagation, and can occur in large numbers on a host during the wet seasons (Walker, 2003). It transmits the protozoan pathogen *Babesia bigemina* that causes

bovine babesiosis, and the rickettsial organism, *Anaplasma marginale* that causes cattle anaplasmosis (Morzaria *et al.*, 1977; Akinboade, 1981). Babesiosis and anaplasmosis are diseases that can run acute or chronic courses in infected livestock, resulting in reduced production, poor growth rates, anaemia, abortions and even death. Dairy breeds of cattle are more susceptible to the diseases relative to the indigenous zebu breeds (Mwangi *et al.*, 1998).

Topical application of chemical acaricides on cattle, either through plunge dips, manual or mechanized spraying, or others as pour-on and spot-on emulsions, are the main modes used conventionally to control the tick for improved productivity (Nari, 1990).

1.2: STATEMENT OF THE PROBLEM

Tick control practices are technical operations that require adherence to regulations on frequencies of livestock dipping, types of acaricides to be used and dilution rates to sustain their efficacies. The statutes in Kenya recommend acaricides for use; prescribe their dilution rates for dipping, modes and frequencies of application on livestock. Nandi County dairy cattle industry depends on the use of chlorfenvinphos, amitraz and alphacypermethrin acaricides, which have been used in dipping for more than one decade, to control ticks. The weekly dipping regime stipulated for acaricide application in dairy zones is suitable for control of three-host ticks, as *Rhipicephalus appendiculatus* (Neumann, 1901), but offers favorable selective pressure for those with fewer hosts. One such tick is the one-host *R. (B) decoloratus*. Other practices, as length of time an acaricide is used on a population of ticks, dip-wash concentration levels and efficiency of monitoring systems are essential for reduction of tick populations and tick-borne diseases. The records that accrued from tick control services in the County were archived without analysis for performance or compliance

with the regulations. Reported persistence of *R. (B) decoloratus* on dipped cattle may be due to poor management of acaricides, their prolonged use tick tolerance and development of resistance to acaricides in use.

1.3: JUSTIFICATION

Development of animal resources is enshrined by the Fourth Schedule of the Constitution of Kenya (Constitution, 2010). The tick control regulations and enforcement are anchored in the country's Cattle Cleansing Act, a legal provision that was first formulated in 1937, and lately reviewed in the year 2016 (CAP 358, 2016). The Act empowers the State Director of Veterinary Services (DVS) to prescribe different acaricides for use in various Counties, regulate the modes and frequencies of their use, monitor qualities of dip-wash used, collect data on usage and recommend relevant improvement. Prescription of acaricides is a strategy, also called zoning that restricts specific acaricides to different tick-control regions. Zoning reserves some acaricides for future use in Counties upon establishment of tick resistance to the acaricides in use. The zoning plan was designed for acaricide rotation to control the three-host ticks, with assumption that others, such as *R. (B). decoloratus* would be controlled concomitantly (Yegon, 2009). Despite the zoning, farmers can readily access and even use reserved acaricides, thereby exposing ticks to restricted chemicals. Records from past tick control services need analysis for information and improvement on control of ticks and tick-borne diseases. Assessment on efficacies of the acaricides in use, and exploration on their potential manipulations for enhanced performance on the ticks would improve decision-making on investment in the dairy sector for food security and scientific interests in management of *R. (B). decoloratus*.

1.4: RESEARCH OBJECTIVES

1.4.1: GENERAL OBJECTIVE

The study was designed to analyze tick control data held in Nandi County, assess effectiveness of chlorfenvinphos, amitraz and alphacypermethrin acaricides that are

used in the County, and synergy of their mixtures, on the one-host *R. (B). decoloratus* adults and their larvae.

1.4.2: SPECIFIC OBJECTIVES

- i) To analyze existing County tick control data for cattle dipping trends, acaricide concentrations used and numbers of tick-borne disease incidences in the years 2000 to 2012,
- ii) To determine *in vitro* efficacies of chlorfenvinphos, amitraz and alphacypermethrin on adult *R. (B). decoloratus*, collected from dipped cattle, and their larvae,
- iii) To assess synergy of chlorfenvinphos-amitraz, chlorfenvinphos-alphacypermethrin and amitraz-alphacypermethrin mixtures made at 50-50% of their dipping concentrations on adult and larval stages of *R. (B). decoloratus* ticks.

1.5: HYPOTHESES

- i) The tick control services in the County did not conform to the recommended routine dipping of cattle, using standard concentrations of acaricides, for effective tick and tick-borne disease control in the years 2000 to 2012,
- ii) Chlorfenvinphos, amitraz and alphacypermethrin are not effective in control of the one host *R. (B) decoloratus*,
- iii) The efficacies of chlorfenvinphos-amitraz, chlorfenvinphos-alphacypermethrin and amitraz-alphacypermethrin combinations, made with 50-50 mls of the dipping concentrations of each acaricide, on adult *R. (B). decoloratus* and their larval stages, are not different from the single acaricides.

CHAPTER TWO

LITERATURE REVIEW

2.1: THE LIVESTOCK INDUSTRY

The Kenya dairy industry started in early 20th century, with introduction of exotic dairy breeds of cattle to newly-established large scale farms in the Kenya highlands (Ogendo, 1971); and subsequently spread to fast-growing adjacent smallholder farms (Muriuki, *et al.*, 2001). Ticks and tickborne diseases became a challenge to the dairy cattle, leading to the introduction of plunge dips for topical application of chemical acaricides for their control (Lawrence, 1992; Perry, 1992). Emergence of tick resistance to the arsenic acaricide that was widely used became a constraint, leading to rise in tick-borne disease epizootics and economic risks to the industry (Shaw, 1969; Graham & Hourrigan, 1977).

The livestock sector plays a role in food securities and economies of most countries in the world, more so to the estimated half a billion of the world's extremely poor people who depend on livestock for part of their livelihoods (Catley, 2008).

Livestock are a source of food, income, employment and foreign exchange in many developing countries and also serve as a store of wealth, provide draught power, means of transport and source of manure for crop production (Upton, 2004).

Other livestock roles include various forms of insurance to food security, contribution to gender equality, source of business opportunities, recycling of waste products and residues from agricultural industries, avenue for industrialization and poverty alleviation amongst many others (FAO, 2009). Approximately 75% of the world's poor live in rural areas, and agriculture can be one of the means for their poverty alleviation. The highest concentration of such people is found in South East Asia and

Sub-Saharan Africa, where they were estimated to be 400 million and 228 million respectively (The World Bank, 2009).

Livestock have been estimated to contribute one-third of the value of global agricultural output in developing countries and more than half in the industrialized ones (Bruinsma, 2003; Upton, 2004). Investments in the livestock industry have high and sustainable returns for invested capital (Otte, 1997).

The demand for livestock products in world economies improves with economic growth of the countries. This was observed in the fast-developing countries of China, India and Vietnam, whose Gross Domestic Products (GDPs) grew rapidly in the years 1990-2005, with increased demands for livestock products (The World Bank, 2007). The growth improved *per capita* incomes for large populations, with resultant change in food preferences (Delgado *et al.*, 1999). The same phenomenon was experienced in Brazil and Indonesia where GDP growth led to increased demand for livestock products (Wolmer *et al.*, 2005). This was attributed to cultural diversification in diets of consumers as a result of improved incomes (ILRI, 2000).

Kenya's Economic Review of Agriculture (ERA 2015), showed that the country's economy was based on agriculture, which contributed about 26% of the country's GDP and employed about 75% of the population. The sector was a major source of revenue with agricultural produce exports accounting for nearly two-thirds of the total domestic export (ERA, 2015). Kenya is known to be with a well-developed dairy industry relative to other East African countries, and each citizen is estimated to consume an annual per capita of 80 – 100 liters of milk. This consumption, which grows at 2 to 3% annually, is the leading in Africa and is due to outstrip the country's milk production (FAO, 2011).

The National Livestock Development Policy of Kenya (2008) indicates that the livestock sub-sector in the Agricultural sector accounted for 10% of the country's entire GDP, 42% of the Agricultural GDP and employed 50% of the agricultural sector labour force.

A joint study by the Intergovernmental Authority on Development (IGAD) Livestock Policy Initiative and the Kenya Bureau of Statistics (KNBS) in 2011 demonstrated that the livestock contribution to Kenya's agricultural GDP was even larger than the official estimate for the year 2009. It was found that the livestock sector actually contributed more than Kenya shillings (KShs) 318.971 billion to the country's agricultural GDP in 2009, compared to hitherto estimates of KShs 127.723 billion that had been made by the Kenya Bureau of Statistics for the same period. The study showed that milk was the country's most important ruminant livestock product at 4,780,620,000 liters, valued at KShs 257.811 billion, translating to about 70% of the total gross value of livestock's contribution to the agricultural sector. Out of this, cattle milk was valued at Kshs 197.018 billion (Behnke *et al.*, 2011).

The Economic Survey (2015) indicated that Kenya produced 3.4 billion liters of raw milk worth Kshs 154 billion in 2014, out of which 541 million liters was processed.

The dairy sector has been found to be the major source of livelihood for the smallholder farmers where it provided income from milk sales and offered viable alternative to traditional cash crops, through regular income when compared to cash crops, such as coffee and pyrethrum, whose income were paid periodically or annually (Omore *et al.*, 1999).

2.2: THE ESTABLISHMENT OF LARGE-SCALE FARMS IN KENYA

The construction of the Uganda railway line started in Mombasa in 1896 and reached Kisumu in 1901, and ultimately Kampala in 1903 (Gunston, 2004). This transport

system opened up the hinterlands of the country, and by 1915, 8242 square miles of land had been alienated along its path, with leases of 999 years, for settler-farmers to establish large scale farms for cash crop production (King & van Zwanenberg, 1975; Morgan, 1963). This was facilitated legally through the Land Acquisition Ordinance of India (1894), which was extended to Kenya, which appropriated all lands within one mile on either side of the rail line as settlement land (Syagga, 2010). More land was appropriated in 1902, through the Crownland Ordinance that converted all land in the Colonial Kenya to be Crown Land, with leases of 99 years (Okoth-Ogendo, 1991). By 1914 nearly 5 million acres (2 million hectares) had been alienated for establishment of large-scale farms in the Kenya highlands (Syagga, 2010). More land was alienated in later years, thereby expanding the acreage of the large-scale farms.

The publication of The Kenya Land Commission Report in 1934 created three categories of land, namely; The Scheduled Areas (also called White Highlands) that had leasehold or freehold titles for European settlers, the Non-Scheduled Areas (also called Native Reserves or Trust lands) for Africans, and the Coast for Arabs (Kenya Land Commission, 1934). This new categorization eventually allocated a total of 7.5 million acres (2.88 million hectares) of land in the country to settler-farmers, who established more large-scale farms in the Scheduled Areas (Syagga, 2010).

2.2.1: THE ESTABLISHMENT OF DAIRY CATTLE INDUSTRY IN THE LARGE-SCALE FARMS OF KENYA

Cattle farming in the large-scale farms started with indigenous breeds that had been captured during punitive wars against the Nandi and Sotik people (Colonial Annual Report, 1907). The settlers found that integration of livestock with crop farming sustained soil productivity, except that the milk yields from indigenous cattle was low

for commercial exploitation, while exotic breeds were prone to endemic diseases (Hills, 1956; van Zwanenberg, 1975).

According to the Colonial Annual Report for the period 1905-6, that was released in 1907, efforts were made to upgrade the indigenous cattle through crossbreeding at the Government Experimental Farm in Naivasha, currently the National Animal Husbandry Research Station, in 1903 (Colonial Report – Annual – no. 519, 1907).

The first high grade dairy cattle were introduced to Kenya from South Africa in 1908, followed by intensive importation of more dairy cattle between 1910 and 1940, resulting in rapid growth of the Settler-farmers dairy sector, and development of formal institutional and organizational framework for milk marketing, breeding and health services (Ngigi, 2005).

The first organized milk marketing started in 1912 as the Lumbwa Cooperative Society in Lumbwa area, currently Kipkelion, in Kericho County, followed by the establishment of Kenya Cooperative Society at Naivasha area in 1925, and then the Nanyuki Cooperative Creamery at Nanyuki in 1928. The three milk marketing bodies were later merged into the Kenya Cooperative Creameries (KCC) Ltd, whose sole objective was facilitation of production, processing and marketing of milk and its products in the domestic and export markets.

Breeding services, through bulls and farm-based artificial insemination (AI) services, were started in 1935, with eventual establishment of the Central Artificial Insemination Station (CAIS) in 1946 for expanded production and distribution of bull semen to users (Conelly, 1998).

Livestock development activities were not extended to the Non-Scheduled Areas, where permanent livestock quarantines were legally enforced through the Fencing of

land holdings and cattle cleansing legislations of 1928 (Colonial Report-Annual no. 1510, 1929).

2.2.2: THE ESTABLISHMENT OF SMALLHOLDER DAIRY FARMS IN KENYA

The concept of liberal land-ownership reforms in Kenya, which led to establishment of smallholder farms in the Non-Scheduled Areas, was developed through the Swynnerton Plan of 1954 to 1959 (Swynnerton, 1954). The Plan addressed the African land problems through reforms on land tenure, consolidation of fragmented holdings, issuance of freehold titles and removal of restriction on commercial agriculture (Bradshaw, 1990). The reforms created smallholder farms for elite Africans in the Reserves as an effort to defuse tensions from economic marginalization and rising nationalism (Wangari, 1991).

The reforms also facilitated upgrading of indigenous cattle herds owned by the majority of African farmers in medium potential agricultural lands, using communal *Sahiwal* bulls that were kept in bull schemes, until 1956 when Artificial Insemination Services were extended to the areas (Swynnerton Plan, 1954).

The abolition of land categorization, which was recommended by the East Africa Commission (1955) eliminated the Scheduled and Non-Scheduled land boundaries, leading to rapid subdivision of the large-scale farms into smallholder units, and adoption of dairy cattle farming in all smallholder farms (East Africa Commission, 1955). Subdivision of the large-scale farms in the former Scheduled Areas were promoted through various Government-sponsored settlement schemes and private land-buying groups (Belashaw, 1964)

2.2.3: IMPORTANCE OF SMALLHOLDER DAIRY FARMS IN KENYA

Most of the milk produced in Kenya is from the smallholder dairy farms, whose acreages range from two (2) to five (5) acres, with an average of one (1) to ten (10) dairy cattle (**Plate 2.1**); which are estimated to contribute between 80 and 91.8% of the total milk production in the country (Omore *et al.*, 1999; Thorpe *et al.*, 2000; Onono, 2012).

To sustain dairy production by the smallholders, the Kenya Government effected a number of changes in the provision of livestock production and marketing services (Muriuki, 2003). These included expansions of disease control and breeding services to cover more smallholder farms in former Non-Scheduled areas and in the new settlement schemes in the former Scheduled Areas (Ithondeka, 2010).



PLATE 2.1: A smallholder farm with six dairy cattle grazing on natural pasture

2.3: CHALLENGES OF TICKS AND TICK-BORNE DISEASES IN DAIRY CATTLE DEVELOPMENT

Introduction of exotic dairy-grade cattle to the large-scale farms in Kenya was faced with production and fatality challenges from ticks and tick-borne diseases. As early as 1901, the brown ear tick *R. appendiculatus* had been recognized to be a major vector for the protozoan *Theileria parva*, the causative pathogen of the fatal East Coast Fever disease (ECF), especially in exotic breeds of cattle. This was observed after an importation of tick-laden (and pathogen-carrying) cattle from Tanzania (then Tanganyika) to South Africa and Zimbabwe (then Rhodesia), which led to contamination of resident ticks that were hitherto free from *T. parva*, with subsequent ECF epizootics in the region (Dolan, 2002).

Challenges from the tick and ECF to the first dairy cattle that had been imported from South Africa in 1908 to Donholm Estate, a large-scale farm that was situated on the south-eastern edge of Nairobi, led to construction of the first cattle plunge dip in Kenya.



2.2: *R. (B) decoloratus* ticks on a cow

2.4: BIOLOGY OF TICKS

Ticks are invertebrate external parasites that belong to the phylum Arthropoda in the class

Arachnida (Walker, 1994) and they constitute a large proportion of organisms in the Order

Acarina (Soulsby, 1982). About 850 species have been identified worldwide (Furman, 1984; Kahn and Line, 2006).

Ticks are ancient parasites, with the oldest record of infestation on an animal being a member of the *Rhipicephalus sanguineus* (Latreille, 1806) group that was retrieved from a dog mummy excavated at El Deir in Egypt, and dated to the period between the 1st century and 4th century AD (Ontranto *et al.*, 2014).

Morphologically, the Acarina are divided into two families, namely the Ixodidae, which are also called hard ticks and the Argasidae or soft ticks; with most of the cattle ticks belonging to Ixodidae family (Soulsby, 1982).

2.4.1: BIONOMICS OF THE IXODID TICKS

Out of the 850-known species of ticks, more than 650 belong to the Ixodidae family (Kahn and Line, 2006). The ticks have a hard, dorsal shield, called scutum, which covers the entire upper surface of males and the anterior third in the larvae, nymphs and females, and their mouthparts are readily visible from the dorsal surface, unlike the argasidae (Walker, 1994).

Seven genera of the Ixodid ticks are economically important in disease transmission in Africa Andrew and Norval, 1989; Bouwknecht *et al.*, 2010), which include *Rhipicephalus*, *Amblyomma*, *Hyalomma*, *Haemaphysalis*, *Dermacentor*, *Ixodes* and *Margaropus* (Walker, 2003).

The old genus *Boophilus* was re-classified as a sub-genus of *Rhipicephalus*, with *Boophilus decoloratus* changing to *Rhipicephalus (Boophilus) decoloratus* (Guglielmone *et al.*, 2009).

Ixodid ticks are mostly field parasites, and are abundant when pastures and livestock stocking rates are improved, due to increased ability to find hosts (Seifert, 1996). Cattle in free grazing systems carry higher tick infestation, and tick-borne diseases, especially in the wet seasons, when compared to those in confinement (Omore *et al.*, 1999). The tick *Amblyomma variegatum* is a vector for *Ehrlichia* (formerly *Cowdria*) *ruminantium*, which causes heartwater in cattle (Soulsby, 1982).

Ticks are active throughout the year in the tropical environments, as evidenced by serological studies in Western Kenya highlands over a twenty-three months period by Okuthe *et al.*, (2006), who established the presence of sero-positive cases of the tick-

borne diseases, babesiosis, anaplasmosis and theileriosis during both the dry and wet seasons; an indication of the continuous activity of tick vectors throughout the period. Ixodids can survive for long periods in pastures without feed or water due to their various behavioral adaptations that enables them to conserve energy and water as a means of extending life for months and even years in adverse environments as they wait for hosts (Williams, 1986). Tick eggs are more susceptible to adverse climatic conditions while engorged larvae are most hardy stage to adverse climatic conditions (Short *et al.*, 1989).

2.4.2: Rhipicephalus (*Boophilus*) *decoloratus* (Koch, 1844)

The tick *R. (B) decoloratus*, also known as the African blue tick, is a one-host tropical Ixodid that is referred to as the blue tick due to the bluish colour of the engorged ticks (**Plate 2.3**). It is found in humid areas of Central, Eastern and Southern Africa where it chiefly parasitizes cattle, but may also be found on sheep, goats, equines, wild ungulates and dogs (Walker, 2003).

It is the principal vector to the pathogenic haemoparasites *Babesia. bigemina*, *B. bovis*, *B. divergens* that cause bovine babesiosis (red water) and *Anaplasma marginale* and *Anaplasma centrale* which cause bovine Anaplasmosis. It is also vector to *Borrelia theileri*, which causes spirochaetosis (Walker, 1970; Soulby, 1982; Sonenshine, 1991; Tonnesen *et al.*, 2006). The tick has been demonstrated to have ability to over-winter and transmit the Lumpy skin disease virus, a member of the genus Capripox virus, which causes Lumpy skin disease of cattle. The virus is related to sheep pox virus and goat pox virus (Lubinga, 2013).

The tick is a member of the sub-genus known to be the first in development of resistance to different acaricides, even when used correctly (Walker, 1970).



PLATE 2.3: Engorged female *R. (B). decoloratus* ticks spanning 0.5 -1.0 millimeters in length

2.5: ECONOMIC IMPORTANCE OF *R. (B) decoloratus*

Tick infestation is a major economic constraint to livestock production in the tropics, including sub-Saharan Africa, with their presence being correlated to the presence of the infectious pathogens they transmit (Lorusso *et al.*, 2013).

The larval, nymph and adult stages of *R (B) decoloratus* are avid, obligate blood-sucking parasites that infest and feed on wide range of living terrestrial vertebrates, onto which they attach either through direct contact between infested hosts, or from the free-living larval stages in the environment (Seifert, 1996).

The tick cause anaemia, stress, irritation, allergy, toxicosis, reduced weight gain and production in their hosts (Jongejan and Uilenberg, 2004). It also acts as a reservoir and vector to a wide range of pathogens, which are perpetuated vertically

(transovarial) in populations by passage from infected ticks through their eggs to their larvae (Uilenberg, 1981). Hides and skins from infested cattle produce low quality tanned products due to perforations and fenestrations caused by tick bite wounds (Jongejan and Uilenberg, 2004; Ahmed, 2015).

2.5.1: LIFE CYCLE OF *R (B) decoloratus*

The tick parasitizes one host in the course of a life cycle, and thus classified as a one-host tick (Sonenshine, 1991). It's typical life cycle of the tick consists of egg, larva, nymph and adult stages (Walker, 1970). The eggs hatch into six-legged larvae that moult into eight-legged nymphs (Walker, 1994). It is only the adult stages that show sexual dimorphism (Cupp, 1991).

On attachment to host, larvae feed for about seven days before undergoing a period of quiescence to moult into nymphs, which re-attach on same host to feed before moulting to adults. Young adults re-attach on the same host to feed and mate before dropping to lay eggs and die (Vatsya *et al.*, 2006).

Under warm and moist weather conditions, the tick can complete its life cycle on same host within three or four weeks and results in heavy, infestation of hosts (Walker, 2003). Unfed larvae can survive for up to seven months in pastures (Soulsby, 1982).

The males remain on the host and are attracted to new feeding virgin females for mating (Cupp, 1991)

The drop-off of engorged female ticks from their hosts is a physiological process, with a circadian rhythm, timed to coincide with grazing areas that have favorable conditions suitable for post-engorgement development and availability of hosts (Balashov, 1967).

On the ground, the gravid female tick seeks a sheltered spot, either beneath stones, clods of soil, or in crevices of walls and cracks of wood, undergoes a pre-oviposition period of three to ten days, depending on weather conditions, then it lays a batch of about 2,500 small, spherical yellowish-brown to dark-brown eggs and dies (Dipetolu *et al.*, 1991). The eggs hatch into three-legged larvae within 3-6 weeks, subject to the prevailing weather; with warm and moist conditions shortening the life cycle, especially the length of the pre-oviposition period and hatching of eggs (Soulsby, 1982).

2.5.2: Ability of ticks to locate and attach on hosts in pastures

The unfed larvae await their suitable hosts by climbing to the tips of grasses and shrubs, a position known as questing, in the places frequented by hosts (Walker, 1970). The daily vertical migration of the larvae to grass blades is a circadian rhythm influenced occurring as a direct response to the environmental temperature, humidity and solar radiation (Balashov, 1967).

The larvae identify the hosts through detection of the animal's breath, body odours, body moisture, temperature and vibrations, by means of a chemoreceptor-bearing sensory organ called the Haller's organ, found at the terminal segment of the first pair of their legs (Hess *et al.*, 1982). Upon climbing a suitable host, the larvae move to their predilection (preferred) body sites where they cut the skin and attach themselves firmly, within twenty minutes, by means of cement-like salivary substance and backward-facing barbs on the mouthparts (Sonenshine, 1991).

The ticks create a space within the bite site for blood to accumulate, and inject saliva, whose molecules modulate host defense responses to the benefit its feeding (Mans, 2010). The saliva has anti-coagulant properties that keeps blood flowing (Kazimirova *et al.*, 2010), inhibitors of thrombin action to stop blood coagulation (Koh, 2009),

antihistamine to reduce itching on site of the bite and an immunosuppressant to reduce leukocyte attack on the mouthpart (Mori *et al.*, 2010).

During feeding, the tick concentrates nutrients in their guts by pumping out excess water, extracted from the blood meal through pumping mechanisms of their salivary glands, into the host and in process they transmit pathogens (Texas and MUS, 2011).

The pathogens, which are transmitted either from the host to the feeding tick through blood meal or from the tick or the host through the saliva, have evolved intricate techniques to exploit the saliva molecules for their survival and multiplication in the tick, and for transmission to- and establishment in host (Nuttall and Labuda, 2008).

All the feeding tick stages are parasitic, with males feeding less than females but remaining longer on the host and may mate with several other females (Cupp, 1991).

The males are attracted to new virgin females that attach on the host by pheromones emitted by the females (Andrew & Norval, 1989).

Although it is noted that ticks which detach before complete feeding will rarely feed during the same stage, it has been observed that they have capacity to re-attach when their feeding is interrupted before adequate engorgement for development to the next stage ((Soulsby, 1982; Wang *et al.*, 1999). An example of such re-attachment is seen following hair grooming or death of the host (Wang and Nuttall, 2001). Also, the males readily detach and move to new sites on the host to mate with newly-attached virgin females Cupp, (1991).

2.6: TICK CONTROL USING PLUNGE DIPS

The first plunge dip in the world was developed in King's Ranch, Texas, in the United States of America in 1866 (Skaggs, 1973). The first one in Kenya was constructed in Donholm Estate in 1912, to apply arsenic (Sodium arsenate) acaricide to control *R. appendiculatus* ticks on the Ayrshire cattle that had been imported from South Africa;

with widespread dipping expanding to other dairy cattle farms after the second world war of 1939-1945 (Hill, 1956), when less-toxic organochloride and organophosphate groups of acaricides were introduced to replace the toxic arsenic (Seifert, 1986).



PLATE 2.4(a): A plunge dip showing the cattle collection yard and dip tank (Note the branding with Steladone™, one of the trade marks for chlorfenvinphos)



PLATE 2.4(b): A plunge dip showing the cattle drying race and a side-water tank

2.7: POLICY OF KENYA ON PLUNGE DIPPING OF CATTLE

The Cattle Cleansing Ordinance was enacted in 1929 to provide regulations for the control of ticks and tick-borne diseases in the large-scale farms within the scheduled areas.

Widespread adoption of tick control practices through dipping took effect on revision of the Cattle Cleansing Ordinance, that was renamed CAP 208, in 1937 (Hill, 1956).

The majority of the acaricides to be used in Kenya were approved for the control of the brown ear tick, *R. appendiculatus*, at intervals not exceeding seven days (Legal Notices numbers 549 (1968), 1995 (1983), 861(1981), 129 (1973), 212 (2003), 399 (2007) and 46 (2013). All other tick species were, and are still expected to be controlled concomitantly (Yegon *et al.*, 2009).

The review off Cattle Cleansing Act in 1967 introduced compulsory dipping in all dairy farming zones, with acaricides being procured centrally by the DVS for distribution to registered communally-used dips, and technical services provided by personnel in public service employment (CAP 358). The ACT empowered the DVS to approve and prescribe specific acaricides for use in clusters of districts (referred to as zones).

2.7.1: RESTRUCTURING OF TICK CONTROL SERVICES IN KENYA

With advent of independence, an exodus of private service providers and settler-farmers, prompted the new government to offer services through the public sector, as it trained more professionals (Chema and Gathuma, 2004). Direct government intervention through marketing Boards and Parastatal organizations, together with control of production and marketing was considered as a means for improving service delivery and income distribution (Hewitt de Alca'ntara, 1993).

The budgetary requirements for delivery of the services were not economically sustainable, especially in the late 1970s, when there was severe socio- economic challenges posed by oil crisis in the world, with subsequent world economic recession (Tapia, 2013). Kenya responded to the economic difficulties by introducing changes in its development plans, including the Structural Adjustment Programmes (SAPs) that were introduced by the World Bank and the International Monetary Fund (IMF) in the 1980/81 fiscal years (Rono, 2002).

The SAPs were intended to assist developing countries to improve their economies through reduction of the role of State while stimulating growth of competitive and productive private sector for renewed growth (Rono, 2002). The SAPs were integrated into Kenya policy and programmes through the publication of the Session Paper No. 1 of 1986 (GoK, 1986), but implemented from 1988 onwards, leading to

restructuring and liberalization of many Government programmes in the country (Rono, 2002).

The changes included financial sector policy reforms, government budget rationalization, divesture and privatization of parastatals, and civil service reforms. Others were the decontrols of domestic marketing of agricultural commodities and the consumer and producer prices (Central Bureau of Statistics, 1997b).

The SAPs led to privatization of cattle breeding, clinical and dipping services; and stoppage on recruitment of new staff, removal of technical staff from the privatized services and mass voluntary retirement of the non-essential and redundant staff (Ithondeka, 2010).

The Government divesture from management of cattle dips was implemented in 1991 followed by the liberalization of milk marketing in 1992 (Ngigi, 2005).

The private sector could not fully take up the liberalized services, while the users lacked financial resources and skills to sustain them (Chema and Gathuma, 2004). As a result, the Kenya Veterinary Association (KVA) initiated a privatization scheme named the Kenya Veterinary Association Privatization Scheme (KVAPS) in 1994, in attempt to facilitate its members with credit to set up private practices, but achieved limited success (Chema and Gathuma, 2004).

In 1998 GoK made effort to salvage the collapsing livestock production services by gradually increasing cost recoveries, encouraging dairy farmers cooperative societies to enter service delivery and encouraging establishment of private veterinary practices to take up the services. The efforts produced practices in areas that were easy to serve, with veterinarians venturing into integrated enterprises engaged in drug sales and on-call clinical and breeding services (Ngigi, 2005).

2.7.2: ACARICIDES USAGE FOR TICK CONTROL

Acaricides are pesticides designed to control the harmful species of Acari group of organisms, such as ticks and mites. They can be applied on cattle, either through plunge dips, mechanized spray pumps, hand pumps, hand dressing, topical pour-on or spot-on pastes, or injected subcutaneously (Nari, 1990). Topical application of chemical acaricides remains a major method used to reduce ticks and tick-borne diseases in livestock and pets (Spickett *et al.*, 1992). Plunge dips offer easy and effective wetting of animals with the acaricides when compared to the use of other conventional methods, but the dip-wash must be maintained at the recommended concentration and the cattle dipped at stipulated periodic intervals (Drummond, 1983). Efficacies of acaricides on ticks depend on their mode of action, quality and quantity of active ingredient deposited on the cattle (George, 2000).

Most acaricides are applied on cattle as wettable powders or emulsions because of their long residual effect on the skin of animals (Seifert, 1996).

2.8: THE CHARACTERISTICS OF STUDY-ACARICIDES

2.8.1: CHLORFENVINPHOS

Chlorfenvinphos is an organophosphate, a group of broad-spectrum acaricides with the general formula, $O = P(OR)_3$, that can kill all tick stages by inhibiting the activity of acetylcholinesterase, the enzyme that breaks down the neurotransmitter acetylcholine that conducts impulses across nerve cell synapses and at the neuromuscular junctions (Taylor, 2001). The inhibition leads to accumulation of the neurotransmitter at the junctions, causing continuous and excessive stimulation and paralysis of target tissues, an occurrence that eventually kills the tick (Corbett, 1974).

2.8.2: AMITRAZ

Amitraz is a formamidine, with the simple formula as HC(=NH)NH_2 , was introduced as an acaricide in 1971 to control *R. (B). microplus* (Stone, 1974). It has fast action on ticks and remains effective for at least seven days on the animal's skin, where it diffuses widely by dissolving in skin oils, to reach parts that were not wetted by the dipping application (Seifert, 1996).

The acaricide is widely used to control ticks on cattle at dipping concentration of 0.025% (Haigh and Gichang, 1980; Davey *et al.*, 1984). It kills the ticks by blocking receptors for the neurotransmitter octopamine in the peripheral and central nervous system and also by inhibiting the synthesis of the enzyme monoamine oxidase in the nerve junctions, an interference that leads to accumulation of the octopamine, resulting in over-excitation of the nerves, paralysis of the tick and death (Dudai *et al.*, 1987).

Apart from killing ticks, the acaricide detaches them from hosts by interfering with their metabolism, reducing glycogen and glucose levels, and by interfering with the respiratory enzyme systems through blockage of the NADP-fumarate reductase enzyme (Seifert, 1996).

On contact with amitraz, ticks lose their proprioception and memory abilities (Hollingworth, 1982) and detach prematurely with loss of capacity to re-attach (Roulston *et al.*, 1971). About 90% of susceptible ticks drop off between thirty (30) minutes and eight (8) hours from exposure, and those that detach without dying would fail to lay eggs or produce less with as low as 0-2% hatching rates (Kagaruki, 1996). At low concentrations (0.0014%), the acaricide has been found to be kill 99% (LC99) of *R. (B) decoloratus* (Kagaruki, 1996).

2.8.3: ALPHACYPERMETHRIN

This is a synthetic pyrethroid, with the structural formula $C_{22}H_{19}Cl_2NO_3$, that kills ticks by acting directly on the excitable membranes of their peripheral and central nerve neurons (Casida *et al.*, 1983). The effect keeps the sodium channels on the neuron continuously open, thereby producing an intensive and repetitive depolarization of the axon, with subsequent paralysis and death of the organism (Tan, 2002).

2.9: EFFECT OF PROLONGED USE OF AN ACARICIDE ON TICKS

Acaricides are formulated as wettable powders for prolonged presence on the coats of animals, and their dilutions produce emulsions (Seifert, 1996).

Apart from their killing effect, acaricides break the life cycles of ticks by preventing their full engorgement, limiting egg production and hatchability (Haque *et al.*, 2014).

Prolonged or frequent use of acaricides offer selective advantages to ticks that have innate ability to withstand the effects of the acaricides, especially in the one-host ticks, that can complete one life cycle in twenty-one days and three to five generations annually (George, 2004). The ticks *R. (B). microplus* and *R. (B). decoloratus* that resist different classes of older acaricides as organochlorides and organophosphates among others, have been found in Southern Africa (George, 2004). However, tick response to under-strength dip-wash has been found to be similar to their response to low level resistance or early stages of high-level resistance (Baker *et al.*, 1965).

Emergence of resistance in field is also influenced by acaricide management, especially use of sub-optimal concentrations and compounds with long residual effects (Seifert, 1996). The weekly dipping frequency used in Kenya was designed to control the three-host *R. appendiculatus*, which has various life cycle stage of the

population on the ground, but encourages faster selection for resistance in the one-host *R. (B). decoloratus* (Wharton, 1976).

The rate at which resistance develops for any acaricides depends on the modification of target sites, initial frequency of initial resistance genes in the tick population, increased ability of the ticks to destroy it through metabolism or sequestration, intensity of the selection, and the ability of the ticks to reduce acaricide penetration through their outer protective layers (Noppun *et al.*, 1989). Resistant ticks can spread among herds of cattle through livestock movement, as was seen in Yucatan State of Mexico, which occurred at a prevalence estimate of 19.4% (Jonsson *et al.*, 2007).

A study in Natal, South Africa, found that field strains of *B. decoloratus* developed resistance to the synthetic pyrethroid fenvalerate only after eighteen months usage (Coetzee *et al.*, 1987).

The onset of resistance to acaricides may be delayed through the usage of acaricide as recommended by manufacturers, destruction of all ticks in treated animals, usage of recommended compounds until the regulatory authority advises on change, and reservation of new acaricide products for use when the older ones become ineffective (Latif, 2004).

Resistance is common among *B. microplus* and *B. decoloratus*, with the resistance commonly occurring within five to ten years of the introduction and routine application of an acaricide (Wharton, 1983). This is due to its short life cycle where more generations are produced in a short time thereby speeding selection process, and the single-host life cycle that exposes the greater population of the ticks to acaricides with every dipping (Wellcome, 1980).

The development of resistance is the major determinant for the need of new acaricide products (George, 2000).

2.9.1: Impact of resistance on tick control and dairy cattle productivity

The cost of developing new acaricide molecules is prohibitive and resistance may discontinue their usage before developers recover economic returns from their use, thereby discouraging research for new compounds (Durand, 1976).

Some populations of *R. (B). decoloratus* that are resistant to camphechlor, carbaryl, benoxafos, dioxathion, diazinon, quintiophos and carbofenenothion acaricides have been observed in other parts of the world (Cotton *et al.*, 1982).

The first case of tick resistance in Kenya was noticed in 1953 when *R. (B). decoloratus*, survived dipping with arsenic (Ongare, 1982). Confirmed resistance by the tick to amitraz was observed by Yegon *et al.*, (2009) in Sugoi area of Uasin Gishu County.

2.10: COMBINED ACARICIDES

Combinations of fibronil and permethrin were found to be synergistically effective against the ticks, *Dermacentor reticulatus*, *Ixodes ricinus* and *R. sanguineus* on dogs (Jongejan *et al.*, 2015; Dumont *et al.*, 2015), while those of amitraz and cypermethrin were effective against *R. appendiculatus* and *R. (B) decoloratus* infesting buffalos in South Africa (Van de Merwe *et al.*, 2005). Studies on combinations of thymol, carvacrol and cinnaldehyde were established to have high efficacy on larval stages of *Amblyomma sculptum* (Daemon, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1: STUDY AREA

The study was conducted in Nandi County (**Figure 3.1**), which is located in the North Rift Region of Kenya. Geographically, it has the equator as the southern boundary and extends northwards to latitude 0.034 ° N.; and lies between longitudes 34.045° E and 35.025° E. The County covers an area of 2,884 sq. km. and has a cool, wet climate with temperatures that range from 15°C to 25°C. The rainfall ranges from 1200 mm – 2000 mm per annum, with the long rains occurring between the months of March and June while the short rains start from mid-September to November (Nandi County website).

According to the Kenya Population and Housing Census (2009), the County has a cattle population of 309,039 based in smallholder farms, where they produce milk for household use and sales to traders and processors (DA, 2016).

The *R. appendiculatus* and *R. (B). decoloratus* ticks are endemic to the area (Walker, 1970; Wangila, 2016), and the County government supports the tick control services through the use of communal plunge dips. The support includes dip rehabilitations, provision of acaricides and extension services, coupled with provision of artificial insemination services to improve the quality of the dairy cattle (DA, 2016).

The County practices the weekly dipping policy for the control of *R. appendiculatus*, with expectation for concomitant control of *R. B. decoloratus* and other ticks.

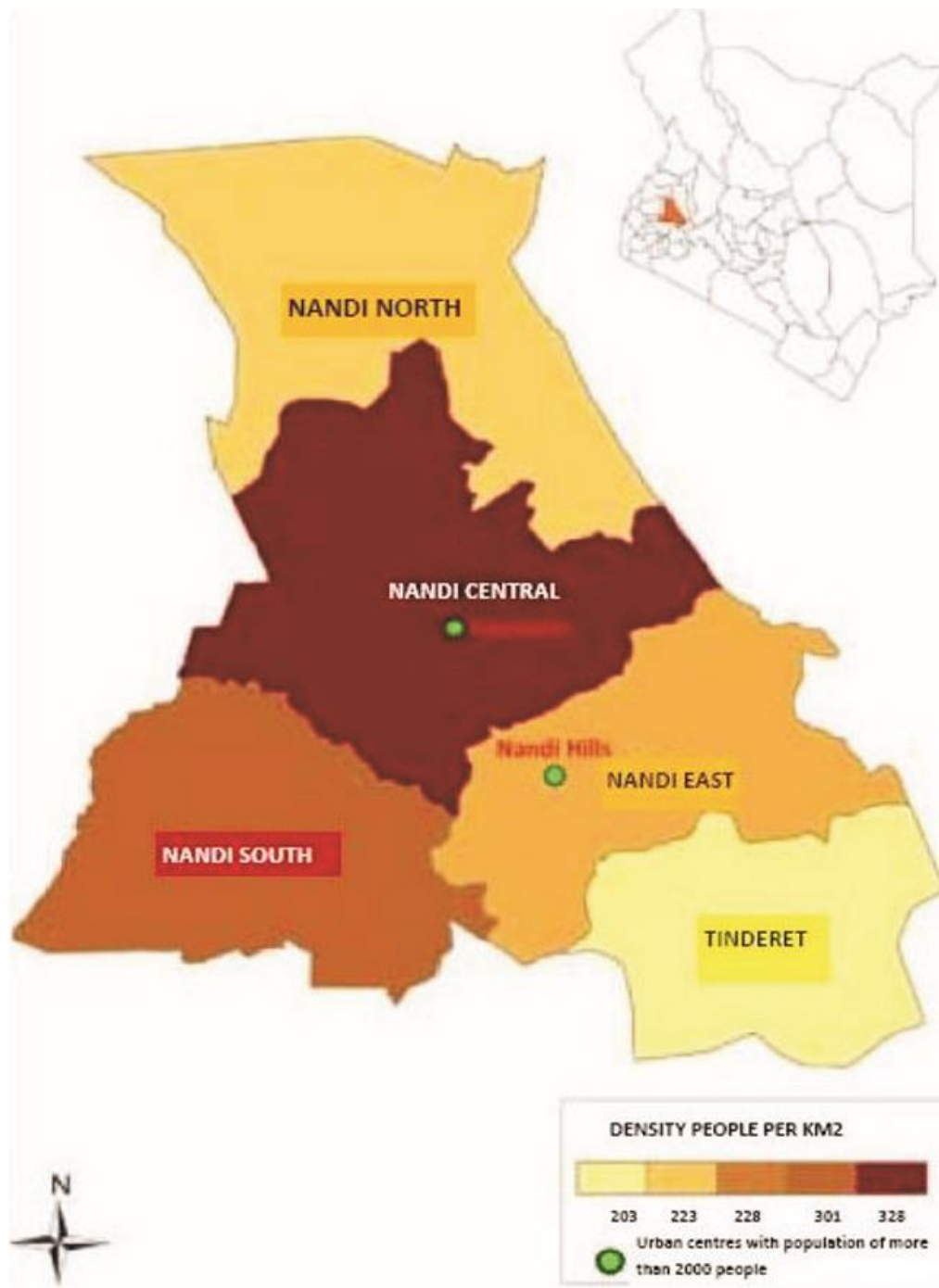


Figure 3.1: Map of Nandi County (Geographic location shown in side-map of Kenya)

Source: <https://www.kenyacountyguide.co.ke/nandi-county-029/>

3.2: MODES OF STUDY

The study was carried out in phases which involved desk studies on County annual reports, collection of adult *R. B. decoloratus* tick from cattle in selected farms, incubation of the ticks for production of larvae, and *in vitro* efficacy tests using the study acaricides and their mixtures on the ticks. The adults were tested through the Adult Immersion Test (AIT), as described by Drummond *et al.*, (1973) and the larvae through the Larval Packet Test (LPT), as described and prescribed by FAO (2004).

3.3: DESK STUDIES

The studies were done through examination of the information in the Tick Control Annual reports for the years 2000 to 2012, held in the County Veterinary Head-Office, in Kapsabet town. The information derived were on the plunge dips, numbers of cattle dipped annually, data on tick-borne diseases, and the concentration status of dip-wash used during the period.

The plunge dips were examined for the functional trends of the communally-managed and the privately-managed facilities, while numbers of cattle dipped were examined for their annual trends. Annual incidences of the tick-borne diseases anaplasmosis, East coast fever, babesiosis and heart water were examined for their trends while the dip-wash records were examined for trends under the under-strength, normal (standard) and over-strength concentration classes.

3.4: IDENTIFICATION OF CATTLE FOR TICK COLLECTION

The ticks were collected from cattle in Kapsabet sub-county, where persistent ticks had been reported. The functional dips that used the study-acaricides were listed, and three dips randomly chosen for each acaricide. Five farms that had dipped their cattle three days prior to the study were randomly identified from their dipping registers of

the selected dips, and visited for tick collection. Ideally, susceptible ticks die or fall off host within one day from dipping, and no adults would be expected on the cattle on the third day. All adult cattle in the chosen farms were sampled for the ticks. The services of three motorized Animal Health Technicians were engaged to collect visibly engorged *R. (B). decoloratus* from the cattle in the identified farms.

3.5: COLLECTION AND HANDLING OF ADULT TICKS

The ticks from cattle that were dipped with similar acaricides in a sub-county were carefully picked into labelled vials and taken to the laboratory at the University of Eldoret for overnight storage at room temperature. On the following day, the batches of ticks that were exposed to similar acaricides in the three sub-counties were pooled in labelled basins and washed with water. Gentle whirling with a plastic spatula was used to mix the ticks in process of washing, before drying them on blotting paper. The ticks in each acaricides category were then weighed individually, using the Electronic Balance Type UV 420H, to establish their feeding weights in spite of the dipping.

From each of the three tick groups, eight (8) tick sub-groups, each with twelve ticks for the amitraz and alphacypermethrin dips, and three ticks in the chlorfenvinphos dips, were randomly selected, in two replicates and placed in labelled jars as follows:

Chlorfenvinphos dip category sub-groups: C1, C2, C3, C4, C5, C6, C7, C8.

Amitraz dip category sub-groups: A1, A2, A3, A4, A5, A6, A7, A8., and

Alphacypermethrin dip category sub-groups: CP 1, CP 2. CP3, CP4, CP5, CP 6, CP 7, CP 8.

3.6: PREPARATION OF TEST-ACARICIDES

Commercial stocks of test acaricides were procured and freshly diluted with water, as per manufacturer's instructions for tick control, for efficacy tests on ticks at the recommended concentrations. Binary combinations of the dilutions were also made

by mixing equal volumes of the diluted acaricides, to produce the combinations, chlorfenvinphos-amitraz, chlorfenvinphos-alphacypermethrin and amitraz-alphacypermethrin mixtures.

The dilutions and their mixtures were tested for their efficacies on adults and larvae.

3.7: TESTING OF THE ADULT TICK SUB-GROUPS

The test intended to assess whether the acaricides, applied on the ticks at recommended dilution rates for dipping, still had an effect on *R. (B). decoloratus* found on cattle despite the length of usage. The AIT involved direct immersion of adult tick samples, in thirty (30) milliliters of test acaricides in petri dishes, for five (5) minutes as described by Drummond *et al.*, (1973) and modified by FAO in 2004.

The controls were similarly immersed in plain water. The ticks in the sub-groups C1, A1 and CP1 were immersed in the diluted chlorfenvinphos while those in sub-groups C2, A2 and CP2 were immersed in amitraz, and the sub-groups C3, A3 and CP3 in alphacypermethrin.

Ticks in sub-groups C4, A4 and CP4 were immersed in chlorfenvinphos-amitraz mixture while those in sub-groups C5, A5 and CP5 were immersed in chlorfenvinphos-alphacypermethrin, and sub-groups six (6) in the amitraz-alphacypermethrin.

The sub-groups C7, A7 and CP7 were immersed to water as controls.

After the immersions, the ticks were placed in labelled petri dishes for observation at room temperature.

3.8: RAISING AND TESTING OF THE LARVAE

The ticks in the sub-groups C8, A8 and CP8 were put in vials and incubated for seven days under high humidity at temperature of 27°C to lay eggs. The dead tick carcasses were carefully removed from the vials and eggs incubated to raise larval.

Samples of the larvae were placed in different secured packets made with Whatman Filter Paper No. 1, with diameter of 125 mm. The packets were immersed in the test acaricides for five (5) minutes as described by Stone and Haydock (1962) and modified by FAO as the Modified Larval Packet Test (MLPT) (FAO, 1984), and then transferred to dry trays for observation, under room temperature, for mortalities. The observation was done once due to high numbers of casualties within 24 hours from immersion.

3.9: DATA COLLECTION

After the immersions, mortalities of the adult ticks and larvae were judged by absence of body and limb movements.

The numbers of dead adults were recorded in three consecutive readings at 24 hours intervals. A fourth reading after 96 hours was done due to minimal efficacies in the first 72 hours. The mortalities of larvae were recorded once due to virtual elimination of their populations within 24 hours.

3.10: DATA ANALYSIS

The ability of ticks to survive dipping and continue feeding, were assessed through live ticks recovered and live weights.

The efficacy tests were analyzed using Analysis of variance (ANOVA), using Stata 13.1 statistical package and Abbott's Formula (Abbott, 1925), which uses the principle below:

Percent mortality = (Mortality of treated - Mortality of controls) / (Mortality of controls) x100

Tests for normality in sample distribution were tested using the Shapiro-Wilk Test statistics, whose median value, also called the appealing index, V , for normally distributed populations is 1. The V - values greater than 1, would indicate non-normality. The inferential statistics Levene's Test on homogeneity of variances, which tests the assumption that variances across the sampled population groups were equal, was used to assess the equality of variances. Bonferroni Post hoc test was used to re-analyze the data for confirmation of where the differences occurred between the groups.



PLATE 3.1: Eggs laid by *R. (B) decoloratus* in incubation vials (They appear as dark clusters at the bottom of the vials)



PLATE: 3.2 : Packets containing larvae of *R. (B) decoloratus* ready for Larval Packet Test

CHAPTER FOUR

RESULTS

4.1: TRENDS IN FUNCTIONAL STATUS OF DIPS

The study established a pattern of trends in the functional dips. The communally-managed dips (CD) were operated through user-committees as public utilities, while the privately-managed dips (PD) were operated by individual farmers within their farms. The majority of the dips (84.11%) were operated communally while 15.89% were under private management. The functional communal and private dips were 84.85% while the non-functional ones were 15.15%. As presented in **Table 4.1**, an annual average of 286 CDs were in operation during the period, while an average of 45 of them were non-functional. Likewise, 48 PDs were functional while 15 others were non-functional. In summary, 72.76% of the CDs functioned, while 11.35% were non-functional. On the other hand, 12.09% PDs functioned, while 3.8% of them were dormant.

Table 4.1: Summary statistics of functional and non-functional dips under different management systems in the period 2000-2012

Dips	Total	Mean no.of dips	Std. Dev.	Percentage
Communally-managed functional	3718	286.00	20.73644	72.76%
Communally-managed non-functional	580	44.62	16.20462	11.35%
Privately-managed functional	618	47.54	13.46363	12.09%
Privately-managed non-functional	194	14.92	7.857709	3.8%
Total	5110			100%

Observations on **Figure 4.1a** indicated that the number of functional communally-managed dips (CDF) retained a fairly constant trend in the 2000 – 2007 period, followed by a rise from 2008, with peak in the years 2010 and 2011. The functional privately-managed dips (PDF) retained a constant trend between 2000 and 2003, but decreased between the years 2004 and 2012. The non-functional communal dips (CDNF) had alternating trend in the period, while the non-functional private dips (PDNF) rose between 2007 and 2012.

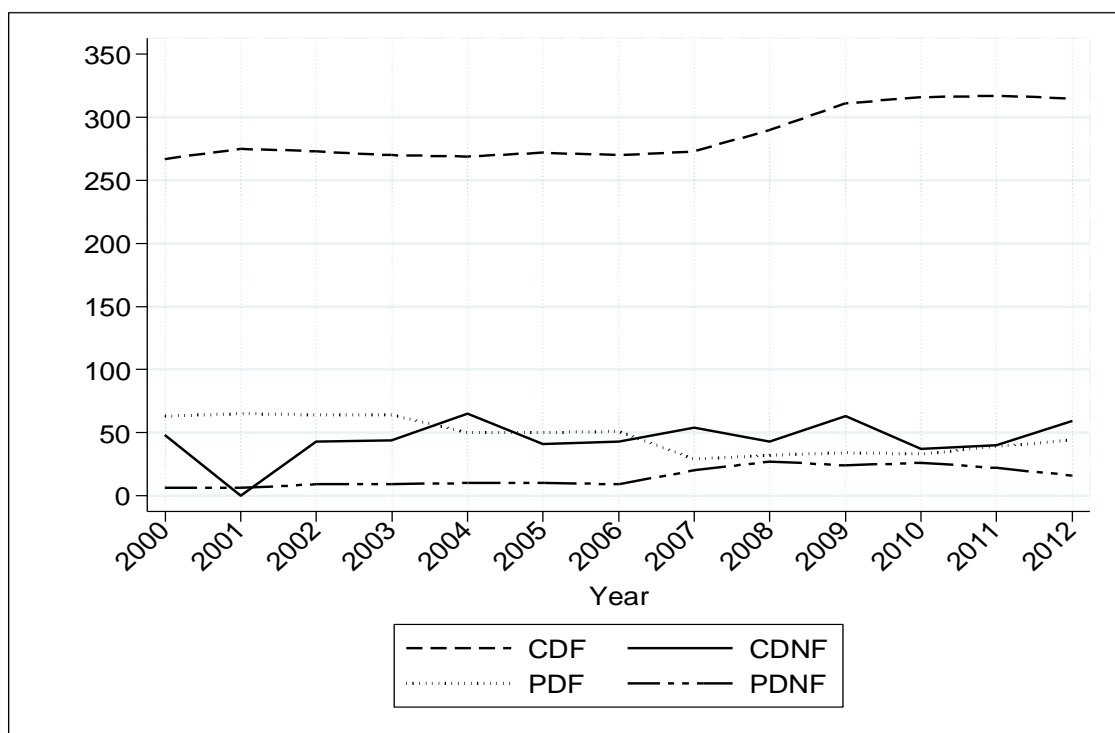


Figure 4.1a: Dip numbers under different management over years 2000-2012

The average number of cattle registered for dipping in the county for the period were $266,178.3 \pm 30,963.01$.

The numbers of cattle presented for dipping annually had a trend as shown in **Figure 4.1b**. The observations indicated that the annual dipping fluctuated in an alternating fashion from 2000 to 2009. However, a steady increase in the annual dipping trend

occurred between the years 2009 to 2012, correlates with the increase in numbers of functional communal dips, shown in **Figure 4.1a**. The lowest number of cattle dipped occurred in 2002, while the highest was in 2012.

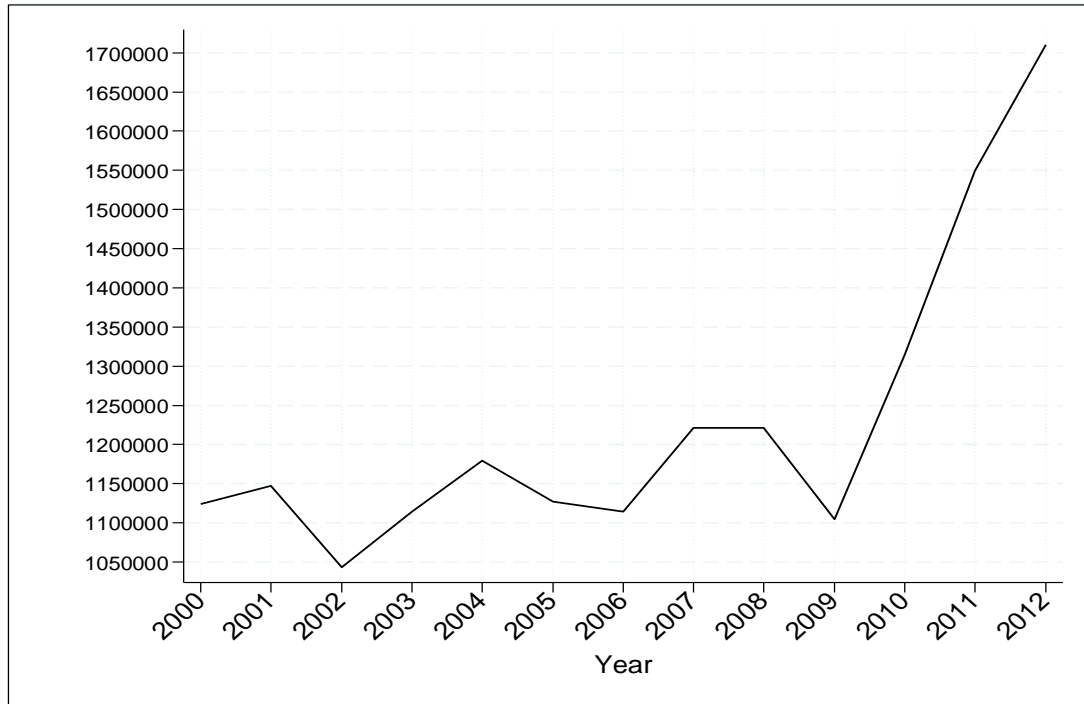


Figure 4.1b: The trend in cattle dipping

A total of 41,767 tick-borne disease cases were recorded between the years 2000 and 2012, with the average being 3,212.85 cases annually (**Table 4.2**). Anaplasmosis was the leading tick-borne disease, with a mean of 1,533 (47.74%) cases annually; followed closely by East Coast Fever at 1,512 (47.08%) cases. Babesiosis and Heartwater had low incidences, with the annual averages of 146 (4.55%) and 20 (0.63%) cases, respectively.

Table 4.2: Tick-borne disease incidences in the 2000 - 2012 period

Disease cases	Annual cases	Std. Dev.	Total incidences	Annual percentage
ECF	1512.615	176.8095	19,664	47.08%
Ana	1533.769	233.3564	19,939	47.74%
Bab	146.3077	33.99114	1,902	4.55%
Hw	20.15385	7.914608	262	0.63%
Total	3,212.85		41,767	100%

Key: ECF = East Coast Fever, Ana = Anaplasmosis, Bab = Babesiosis, Hw = Heartwater

High incidences of Anaplasmosis occurred in the years 2001 and 2009 while ECF was more prevalent in 2006, 2007, 2011 and 2012 (**Figure 4.3**). The least number of ECF cases was recorded in 2009 compared to all other years. Babesiosis and heartwater cases retained a low profile throughout the period, with the latter being the least prevalent.

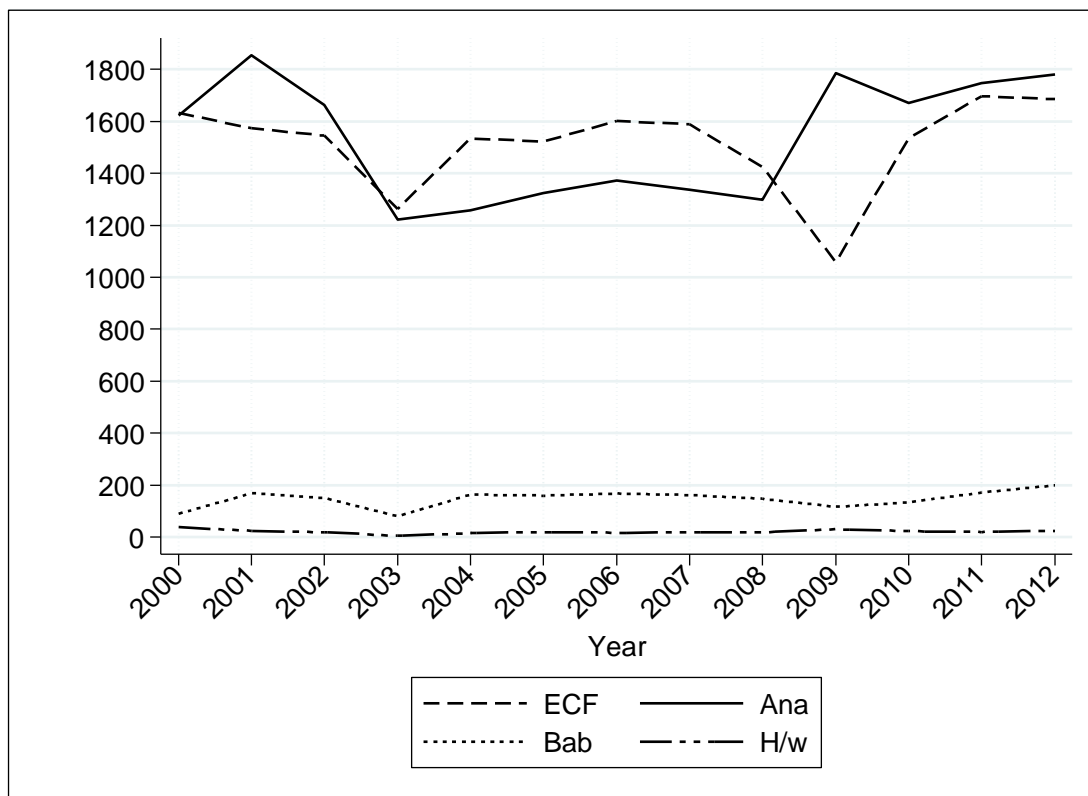


Figure 4.3: Incidences of tick-borne diseases

Out of the 13,208 dip-wash samples presented for analysis in the period, 7,444 (56.36%) conformed with the recommended dipping concentrations (Normal), while 638 (4.83%) had higher concentrations than that recommended (Over-strength). A total of 5,126 (38.81%) samples had concentrations that were lower than the recommended levels (under-strength), as presented in **Table 4.3**.

Table 4.3: Summary of acaricide concentrations in dip-wash samples

Concentration status	Total samples	Mean	Std. Dev.	Percentage
Over-strength	638	49.07692	36.42907	4.83%
Normal	7444	572.6154	114.2859	56.36%
Under- strength	5126	394.3077	154.3855	38.81%
Total	13,208			

The numbers of dip-wash samples with different acaricide concentrations fluctuated in the period (**Figure 4.4**). The highest number of over-strength dip-wash samples were recorded in 2001 and 2008, while the highest numbers for under-strength dip-wash were recorded in 2000 and 2007. However, the trend on numbers of over-strength dip-wash samples decreased from 2008 to 2012.

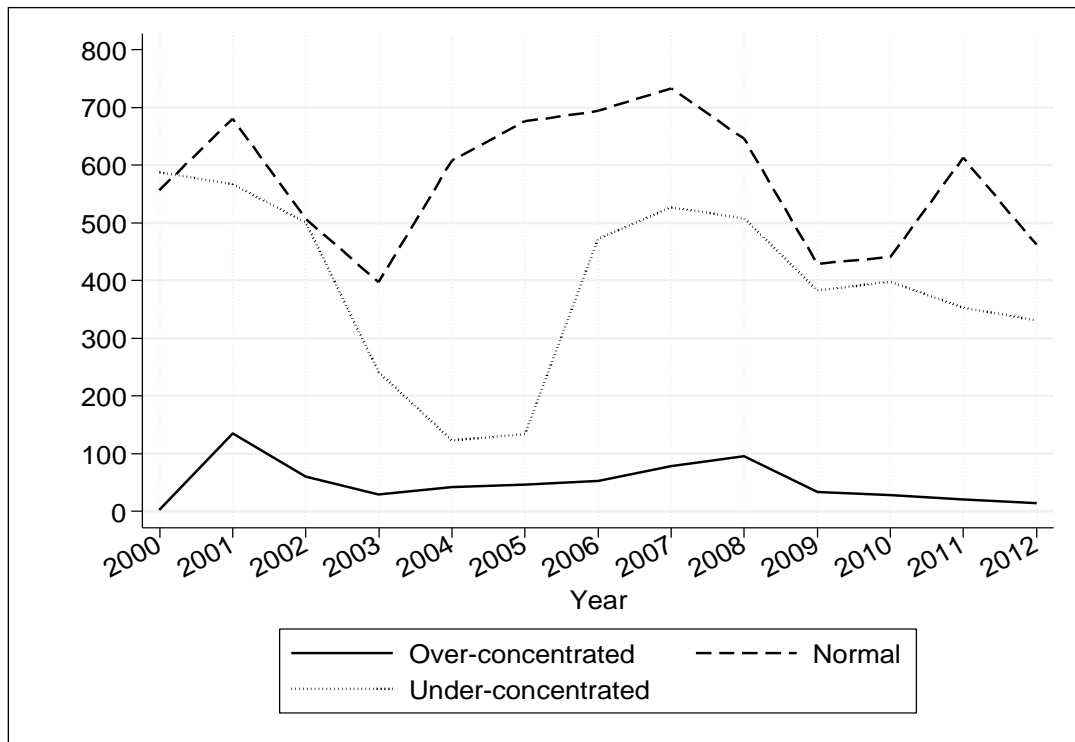


Figure 4.4: Summary of dip-wash qualities used in the period 2000 -2012

4.2: THE EFFECTS OF DIPPING ON *R. (B). decoloratus* TICKS

Numerous active ticks were recovered from cattle despite dipping with either of the acaricides three days earlier (**Table 4.4a**). A total of 398 ticks were collected from 145 cattle that had been dipped with alphacypermethrin, while 321 were recovered from 124 cattle dipped with amitraz. The 118 cattle dipped in chlorfenvinphos yielded 60 ticks. The average weight of the ticks across the three acaricides categories varied, with ticks from cattle that used chlorfenvinphos being heavier, with mean weights of 0.12403g. Ticks from the cattle that used amitraz dips had a mean of 0.1182679g, while those from the Alphacypermethrin dips had 0.1135151g. The presence of active ticks on the cattle indicated that the acaricides used could not eliminate them.

Table 4.4a: Numbers and weights of the collected ticks from cattle

Type of Dip	Ticks (cattle)	Mean weights (g)	Std. Dev.	Min	Max
Alphacypermethrin dip	398(145)	0.1135151	0.0566916	0.01	0.32
Amitraz dip	321(124)	0.1182679	0.521393	0.009	0.243
Chlorfenvinphos dip	60 (118)	0.1240333	0.0528852	0.019	0.258

(Figures in parenthesis show the number of cattle examined)

A test for normality in distribution of the tick samples using the Shapiro-Wilk Test indicated that the appealing index, V , for alphacypermethrin and amitraz had median values greater than 1, which indicated that they were not normally-distributed ($p = 0.0000$ and $p=0.0010$), as shown in **Table 4.4b**.

The appealing index V of 0.694 ($p=0.7842$) for chlorfenvinphos was less than 1, which indicated that the tick weights were normally-distributed. The results showed that the ticks that survived either of the acaricides continued to feed on their hosts.

Table 4.4b: Shapiro-Wilk Test for Normality

Type of Dip	Tick samples	W	V	Z	Prob>z
Alphacypermethrin dip	398	0.97620	6.523	4.461	0.00000
Amitraz dip	321	0.98368	3.692	3.076	0.00105
Chlorfenvinphos dip	60	0.98723	0.694	-0.787	0.78421

4.3: ADULT IMMERSION TESTS (AIT) WITH CHLORFENVINPHOS, AMITRAZ AND ALPHACYPERMETHRIN ACARICIDES ON *R. B. decoloratus*

Assessments of the adult *R. B. decoloratus* ticks from the different dip categories did not achieve the 95% (p -value =0.05) efficacy thresholds used for acaricide registrations in Kenya (Table 4.5). Notable efficacies occurred only after 96 hours, with chlorfenvinphos having an efficacy of 91.67% on ticks from the amitraz dipping and 75% on those from the alphacypermethrin dip after 96 hours from exposure. Alphacypermethrin had efficacies of 75% on the ticks from Alphacypermethrin and the Amitraz dips in the same interval.

Table 4.5: Efficacies of single acaricides according to Abbott's Formula

Source of ticks (Dip category)	Test Acaricide	Ticks alive after 24 hours		
		Mean	Std. Dev.	Abbott Efficacy %
Alphacypermethrin dip	Chlorfenvinphos	12	0	0.00%
	Amitraz	11.5	0.71	4.17
	Alphacypermethrin	12	0	0.00
	Control	12	0	0.00
Amitraz dip	Chlorfenvinphos	12	0	0.00
	Amitraz	12	0	0.00
	Alphacypermethrin	12	0	0.00
	Control	12	0	0.00

Chlorfenvinphos dip	Chlorfenvinphos	2.5	0.71	16.67
	Amitraz	3	0	0.00
	Alphacypermethrin	3	0	0.00
	Control	3	0	0.00
Ticks alive after 96 hours				
Alphacypermethrin dip	Chlorfenvinphos	3	0	75
	Amitraz	11	0	8.33
	Alphacypermethrin	4	0	66.67
	Control	12	0	0.00
Amitraz dip	Chlorfenvinphos	1	0	91.67
	Amitraz	12	0	0.00
	Alphacypermethrin	4	1.41	66.67
	Control	12	0	0.00
Chlorfenvinphos dip	Chlorfenvinphos	2.5	0.71	16.67
	Amitraz	3	0	0.00
	Alphacypermethrin	3	0	0.00
	Control	3	0	0.00

Analysis of variance (ANOVA) indicated that there were no significant differences between the mean efficacies of chlorfenvinphos, amitraz, alphacypermethrin and control (plain water) within 24 hours from immersion. (**Table 4.6**). The tests on ticks from Alphacypermethrin and chlorfenvinphos dips categories, had *F*- values of

0.2853, which was greater than the *alpha* 0.05. The result accepted the null hypothesis, thereby indicating that the means were not different, hence equal in the four tests. The *F*- value of the tick samples from amitraz dip was not generated by the system because the sum of squares between (SS_b) and within (SS_w) the groups was zero (0), indicating that there were no conclusive statistics from the sample.

The 96 hours *F*- values of the tick samples from alphacypermethrin and the chlorfenvinphos dips could not be generated because the SS_w was zero (0). Also, SS_b the test acaricides on the ticks from chlorfenvinphos dips was zero (0). Therefore, the results were inconclusive on possible differences in the tick groups after the immersions. Only the tick samples from amitraz dip category, had an *F*- value of 0.00, which indicated presence of statistically significant difference in the mean efficacies of the tests on the amitraz ticks after 96 hours.

Table 4.6: ANOVA comparisons on the mean efficacies of chlorfenvinphos, amitraz, alphacypermethrin and plain water

After 24 hours

Source of ticks (Dip category)	Source	SS	Df	MS	F	Prob >F
Alphacypermethrin Dip	Between groups	0.4	3	0.1333	1.60	0.2853
	Within groups	0.5	6	0.0833		
	Total	0.9	9	0.1		
Amitraz Dip	Between groups	0	3	0		
	Within groups	0	6	0		
	Total	0	9	0		
Chlorfenvinphos Dip	Between groups	0.4	3	0.1333	1.60	0.2853
	Within groups	0.5	6	0.0833		
	Total	0.9	9	.1		

After 96 hours

Alphacypermethrin Dip	Between groups	162	3	54.133		
	Within groups	.4	6	3		
	Total	162	9	18.044		
Amitraz Dip	Between groups	225	3	75.2	225.	0.000
	Within groups		6			
	Total	225	9	4		

		.6		60
	Within groups	2	6	0.3333
	Total	227	9	25.288
		.6		88
Chlorfenvinphos Dip	Between groups	0	3	0
	Within groups	0	6	0
	Total	0	9	0

(Level of significance $\alpha=0.05$)

This required another analytical test how the test acaricides differed on their effect on the ticks from amitraz dips. A multiple comparison of means, using Bonferroni Post Hoc Test to identify how the tick groups differed from the amitraz group, indicated statistically significant differences between amitraz and chlorfenvinphos groups (0.000), alphacypermethrin and chlorfenvinphos groups (0.012), alphacypermethrin and amitraz groups (0.000), plain water (control group) and chlorfenvinphos group (0.000), and plain water and alphacypermethrin groups (0.000), as observed after 96 hours from exposure (**Table 4.7**).

Table 4.7: Bonferroni Post Hoc comparison of dead ticks from amitraz dip category in 96 hours by Type of Acaricide

Row mean-	Chlorfenvinphos	Amitraz	Alphacypermethrin
Column Mean			
Amitraz	-11		
	0.000		
Alphacypermethrin	-3	8	
	0.012	0.000	
Plain water	-11	0	-8

0.000 1.000 0.000

A check on the assumption that variances were equal across all the adult tick groups after 24 hours from AIT, using the Levene's inferential statistics, gave an F value for the Levene's test as $W0 = 2.53125$ with p -value of 0.0983, which was greater than $\alpha p = 0.05$. The test accepted the null hypothesis that the variances were equal. This was an indication that the test-acaricides did not produce significant killing-effect on the ticks from either of the three categories at 24 hours from immersion (Table 4.8).

Table 4.8 Levene's Test for Homogeneity of Variances

Category of Dip	Mean	Std. Dev.	Freq.
Alphacypermethrin Dip	11.9	.31622777	10
Amitraz Dip	12	0	10
Chlorfenvinphos Dip	2.9	.31622777	10
Total	8.9333333	4.3464876	30

$W0 = 2.53125$ df (2, 27) Pr > F = 0.0982757

$W50 = 0.50000$ df (2, 27) Pr > F = 0.6120366

$W10 = 0.50000$ df (2, 27) Pr > F = 0.6120366

$W0$ = test of mean, $W50$ = test of median, and $W10$ = test of trimmed mean.

4.4: ASSESSMENT ON THE SYNERGY IN COMBINATIONS OF CHLORFENVINPHOS, AMITRAZ AND ALPHACYPERMETHRIN ON ADULT FEMALE *R. (B). decoloratus*

The acaricide combinations produced tick mortalities after 96 hours from AIT exposures (**Table 4.9**). No mortalities were observed in all tick samples in the Control groups (plain water).

The ticks from the alphacypermethrin dips had a mean of 10 mortalities when exposed to chlorfenvinphos-amitraz, while those exposed to chlorfenvinphos-alphacypermethrin had a mean of 9 dead ticks. A mean of 2.5 deaths was observed on exposure amitraz-alphacypermethrin.

All the 12 ticks from amitraz dips were killed by chlorfenvinphos-amitraz combination, while a mean of 8.5 ticks died on exposure to chlorfenvinphos-alphacypermethrin. A mean of 7 mortalities were observed on exposure to amitraz-alphacypermethrin combination.

The ticks from chlorfenvinphos dips survived exposure to chlorfenvinphos-amitraz tests, while a mean 0.5 mortalities were observed on exposure to chlorfenvinphos-alphacypermethrin and 1 mortality on the amitraz-alphacypermethrin combination.

The chlorfenvinphos-amitraz combination was 100% effective on the tick samples from amitraz dips, and 83.33% on the sample from alphacypermethrin dips, but ineffective (0.00% efficacy) on the tick samples from chlorfenvinphos dip.

The chlorfenvinphos-alphacypermethrin was 75% effective on the ticks sampled from alphacypermethrin dips, 70.83% effective on ticks from amitraz dips, and 16.67% on the samples from chlorfenvinphos dips.

The amitraz-alphacypermethrin mixture had low efficacies, with the highest efficacy of 58.33% seen on ticks from amitraz dips. This was followed by 33.33% on ticks from chlorfenvinphos dip, and 20.83% on ticks from alphacypermethrin dips.

Table 4.9: Efficacies of combined acaricides on adult ticks

Summary of Dead ticks				
After 96 hours				
Source of ticks	Type of Acaricide	Mean	Std. Dev.	Efficacy %
Alphacypermethrin Dip	Control (Plain water)	0	0	
	Chlorfenvinphos-Amitraz	10	1.4142136	83.33
	Chlorfenvinphos- Alphacypermethrin	9	1.4142136	75
	Amitraz-Alphacypermethrin	2.5	2.1213203	20.83
Amitraz Dip	Control (Plain Water)	0	0	
	Chlorfenvinphos-Amitraz	12	0	100
	Chlorfenvinphos- Alphacypermethrin	8.5	2.1213203	70.83
	Amitraz-Alphacypermethrin	7	1.4142136	58.33
Chlorfenvinphos Dip	Control (Plain Water)	0	0	
	Chlorfenvinphos-Amitraz	0	0	0.00
	Chlorfenvinphos- Alphacypermethrin	0.5	0.70710678	16.67
	Amitraz-Alphacypermethrin	1	0	33.33

(Low numbers of ticks were found on cattle that had used chlorfenvinphos dips)

An analysis of variance was used to test whether there were statistically significant differences between the efficacies of the acaricide combinations on the different

categories of ticks after 24 and 96 hours from AIT exposures. The results indicated no significant differences between efficacies in 24 hours from exposure (**Figure 4.10**). However, the results showed statistically significant differences between the acaricide mixtures on ticks from all the three categories of dips after 96 hours, with F values less than $\alpha=0.05$. This indicated that the combinations had higher efficacies on the adults.

Table 4.10: ANOVA comparisons on efficacies of acaricide combinations on adult ticks

After 24 Hours

Type of Dip	Source	SS	df	MS	F	Prob > F
(Source of ticks)						
Alphacypermethrin Dips	Between groups	0	3	0		
	Within groups	0	6	0		
	Total	0	9	0		
Amitraz Dips	Between groups	0.6	3	0.2	0.80	0.5376
	Within groups	6.5	6	0.25		
	Total	2.1	9			
Chlorfenvinphos Dips	Between groups	0	3	0		
	Within groups	0	6	0		
	Total	0	9			

After 96 hours

Alphacypermethrin Dips	Between	189.6	3	63.2	44.61	0.0002
	groups					
	Within groups	8.5	6	1.41666667		
	Total	198.1	9	22.01111111		
Amitraz Dips	Between	228	3	76	70	0.0000
	groups					
	Within groups	6.5	6	1.083333		
	Total	234.5	9			
Chlorfenvinphos Dips	Between	1.6	3	0.53333333	6.40	0.0267
	groups					
	Within groups	0.5	6	0.83333333		
	Total	2.1	9			

A Bonferroni post hoc test was used to specify the ticks that had more mortality with acaricide combinations. Comparisons of means mortalities on the exposure, relative to control groups, indicated statistically significant differences between all the combinations against all tick categories, except for amitraz-alphacypermethrin on ticks from alphacypermethrin dips; and the chlorfenvinphos-amitraz and chlorfenvinphos-alphacypermethrin on ticks from chlorfenvinphos dips. The last three tests had significant values that were greater than $\alpha=0.05$ (Table 4.11).

Table 4.11: Bonferroni Post Hoc comparison of mortalities of tick groups after 96 hours by type of acaricide

Type of Dip	Row-Column Mean	Plain water	Chlorf-Amitraz	Chlorf-Alpha
Alphacypermethrin	Chlorf-Amitraz	10		
		0.000		
	Chlorf-	9	-1	
	Alphacypermethrin	0.001	1.000	
	Amitraz-	2.5	-7.5	-6.5
	Alphacypermethrin	0.309	0.004	0.009
Amitraz Dips	Chlorf-Amitraz	12		
		0.000		
	Chlorf-	8.5	-3.5	
	Alphacypermethrin	0.000	0.091	
	Amitraz-	7	-5	-1.5
	Alphacypermethrin	0.001	0.018	1.000
Chlorfenvinphos Dips	Chlorf-Amitraz	0		
		1.000		
	Chlorf-	0.5	0.5	
	Alphacypermethrin	0.555	0.904	
	Amitraz-	1	1	0.5
	Alphacypermethrin	0.043	0.08	0.804

4.5: ASSESSMENT OF THE SUSCEPTIBILITY OF LARVAE TO ACARICIDES

The larvae of ticks from the three categories of dips were exposed to test acaricides through the Larval Packet Test (LPT) and assessed for mortalities. Their mean mortalities were compared against the survivors using the Abbott's Formula (**Table 4.12**). Chlorfenvinphos was 100% effective on the larvae of all tick groups. Alphacypermethrin was 100% effective on the larvae from the amitraz and alphacypermethrin dips, but 93.94% on those from chlorfenvinphos dips. Amitraz had 81% mortalities as the highest efficacy on larvae from chlorfenvinphos dip ticks, and the least efficacy of 50% on those from alphacypermethrin dips.

Table 4.12: Efficacies of single acaricides on larval stages of ticks

Dip Category	Test acaricide	Mean number of larvae tested	Mean of alive	Efficacies
Chlorfenvinphos Dip	Chlorfenvinphos	66	0	100%
	Amitraz	18	6	81%
	Alphacypermethrin	27	2	93.94%
	Control	34	33	-
Amitraz Dip	Chlorfenvinphos	89	0	100%
	Amitraz	67	12	75.71%
	Alphacypermethrin	47	0	100%

	Control	70	70	-
Alphacypermethrin Dip	Chlorfenvinphos	8	0	100%
	Amitraz	33	11	50%
	Alphacypermethrin	21	0	100%
	Control	23	22	-

(Difference in numbers of larvae were used due to handling exigencies)

One-way ANOVA results in **Table 4.13** confirmed significant differences between the mortalities of larvae of the ticks from amitraz and chlorfenvinphos dip categories, with F values of 0.000, which were smaller than the P -value of 0.05, at 95% confidence intervals. There were no significant differences in mortalities of larvae from alphacypermethrin dip ticks since their F - value was 0.3643, which is larger than P -value of 0.05

Table 4.13: ANOVA comparisons on efficacies of acaricides on larvae

Category of Dip	Source	SS	df	MS	F	Prob >F
Alphacypermethrin in Dips	Between groups	255.375	3	85.125	1.40	0.3643
	Within groups	242.5	4	60.625		
	Total	497.875	7	71.125		

Amitraz Dips	Between groups	6761.5	3	2253.8	4507	0.0000
				3333	67	
	Within groups	2	4	0.5		
	Total	6763.5	7	966.21		
				4286		
Chlorfenvinphos	Between groups	1443	3	481	1924	0.0000
Dips					.00	
	Within groups	1	4	0.253		
	Total	1444	7	206.28		
				57		

A Bonferroni post hoc test was used to identify the groups of larvae that significantly differed in response to the tests, and the results are as presented in **Table 4.14**. There were statistically significant differences between the means of larvae from the control groups (plain water) and those of the test acaricides in the chlorfenvinphos and amitraz dip categories (sig. 0.000), at 95% confidence interval. Significant differences were also seen between amitraz and chlorfenvinphos (sig. 0.002) on larvae from the chlorfenvinphos dip. Other significant differences occurred between amitraz and chlorfenvinphos, and between alphacypermethrin and amitraz (sig. 0.001) on larvae from the amitraz dip.

Table 4.14: Bonferroni Post Hoc for Larvae

	Row mean- Col. Mean	Chlorfenvinphos	Amitraz	Alphacyper methrin
Chlorfenvinphos	Amitraz	5.5		
Dip		0.002		
	Alphacypermethrin	1.5	-4	
		0.240	0.008	
	Plain water	33	27.5	31.5
		0.000	0.000	0.000
Amitraz Dip	Amitraz	11		
		0.001		
	Alphacypermethrin	0	-11	
		1.000	0.001	
	Plain water	70	59	70
		0.000	0.000	0.000

The efficacies of the acaricide combinations on larvae, as assessed through Abbott's Formula, ranged from 95.45% to 100% (**Table 4.15**). The chlorfenvinphos-alphacypermethrin combination was 100% effective on the larvae from the

alphacypermethrin and chlorfenvinphos dip categories. A similar efficacy was observed for amitraz-alphacypermethrin on larvae from the amitraz dip.

Table 4.15: Efficacies of combined acaricides on larvae according to Abbott's Formula

Type of Dip	Type of Acaricide	Mean	Std. Dev.	Efficacy %
Alphacypermethrin Dip	Plain water	22	0	
	Chlorfenvinphos-Amitraz	0.5	0.70710678	97.73
	Chlorfenvinphos-Alphacypermethrin	0	0	100
	Amitraz-Alphacypermethrin	0.5	0.70710678	97.73
Amitraz Dip	Plain Water	70	0	
	Chlorfenvinphos-Amitraz	0.5	0.70710678	99.29
	Chlorfenvinphos-Alphacypermethrin	0.5	0.70710678	99.29
	Amitraz-Alphacypermethrin	0	0	100
Chlorfenvinphos Dip	Plain Water	33	0	
	Chlorfenvinphos-Amitraz	1.5	0.70710678	95.45
	Chlorfenvinphos-	0	0	100

Alphacypermethrin

Amitraz- 0.5 0.70710678 98.48

Alphacypermethrin

One-way ANOVA analysis showed that there were statistically significant differences between the means responses of the larval groups to the acaricide tests (**Table 4.16**). The combined acaricides had significant impact on the larvae from the three categories of dips, as shown by an F value of 0.000.

Table 4.16: ANOVA on efficacies of combined acaricides on larvae

Category of Dip	Source	SS	df	MS	F	Prob >F
Alphacypermethrin Dips	Between groups	704.5	3	234.833333	939.33	0.0000
	Within groups	1	4	0.25		
	Total	705.5	7	100.785714		
Amitraz Dips	Between groups	7280.5	3	2426.83333	9707.33	0.0000
	Within groups	2	4	0.25		
	Total	7280.5	7	1040.21429		
Chlorfenvinphos Dips	Between groups	1570.5	3	523.5	2094.00	0.0000
	Within groups	1	4	0.25		
	Total	1571.5	7	224.5		

All the acaricide combinations had statistically significant difference when compared against the control groups (sig. value = 0.000) at 95% confidence interval (Table 4.17).

Table 4.17: Bonferroni Post Hoc Test on effect of combined acaricides on larvae

	Row mean- Col Mean	Chlorfen- Amitraz	Chlorfen- Alphacyp	Amitraz- Alphacyp
Alphacypermethrin Dip	Chlorfen- Alphacyp	-.5 1.000		
	Amitraz-Alphacyp	0 1.000	0.5 1.008	
	Plain water	33 0.000	27.5 0.000	31.5 0.000
Amitraz Dip	Chlorfen- Alphacyp	0 1.000		
	Amitraz-Alphacyp	-0.5 1.000	-0.5 1.000	
	Plain water	69.5 0.000	69.5 0.000	70 0.000
Chlorfenvinphos Dip	Chlorfen- Alphacyp	-1.5 0.240		
	Amitraz-Alphacyp	-1 0.697	0.5 1.000	
	Plain water	31.5 0.000	33 0.000	32.5 0.000

Key: Chlorfen-Amitraz = Chlorfenvinphos-Amitraz, Amitraz-Alphacyp = Amitraz-Alphacypermethrin,
Chlorfen-Alphacyp = Chlorfenvinphos-Alphacypermethrin

CHAPTER FIVE

DISCUSSIONS

5.1: DIPPING PERFORMANCE

The majority of the Nandi County's plunge dips (84.11%) were the communally-managed category, while 15.89% were under private management. As observed by Hewitt de Alca'ntara, (1993) the communal dips were originally constructed and operated by the Government of Kenya as tick control facilities for smallholder farmers. The constructions were necessitated by the introduction of dairy breeds of cattle to former non-scheduled areas, which had limited tick control facilities, and the subdivision of the large-scale farms in the Kenya highlands, which occurred on advent of the country's attainment of self-government, as noted by Chema and Gathuma, (2004). The privately-managed dips were owned and used by individual farmers, who had large farms, large herds of cattle and resources.

Popularity of dipping may be estimated from the number of dips in active use, relative to the non-functional ones that were at an average of 11.35% for the communal dips and 3.8% for private dips during the study period. Despite the apparent usage of dips, the study showed that an average of $266,178 \pm 30,963$ cattle were registered for weekly dipping in the County. This figure had a deficit of 42,861 cattle, when compared with the County's 309,039 cattle population according to the Kenya Population and Housing Census (2009). The records revealed that average annual dipping ranged from 500,000 to 1,200,000 cattle in the period between the years 2000 and 2009, before and exponentially rose to 1,700,000 cattle in 2012. This finding indicated an irregular presentation of the registered cattle for dipping, which suggests that some cattle were dipped occasionally or not dipped at all. If most of the dipped cattle used communally-managed dips, as they were the majority of the operational

facilities, the irregular usage could be attributed to their low performance, observed by Rono, (2002) and Ngigi, (2005), as an after-effect of reduced Government support as part of Kenya's Structural Adjustment Programmes (SAPs) initiated in 1991. The same effect was observed by Ragwa, (2002) in Maara district, where dipping drastically reduced upon divestiture of tick control services from Government. The exponential increase in dipping from the year 2009, which occurred in tandem with increasing numbers of functional communal dips, may be attributed to efforts from Government to salvage the collapsing services through free acaricide provision subject to dip rehabilitations by users (Chema and Gathuma, 2004). It can be concluded that the rise in numbers of operational communal dips was due to rehabilitations of non-functional facilities to attract the free acaricide issues.

The numbers functional private dips (PDF) remained fairly constant in the period 2000-2004, before a fluctuation for the rest of the study period. The numbers of non-functional private dips (PDNF) remained low between 2000 and 2006, with a rise as from 2007. The observations, especially from the year 2009, where there was an increase in numbers of functional communal dips, suggests conversion of some of the private dips to communal facilities, or an abandonment of their use in preference to Government -supported communal dips. shift imply PDNF coincided with the rise in numbers of CDF, suggesting a shift by farmers to the communal dips. This conclusion would support the observation that farmers preferred communal dips, as noted by Drummond, (1983) about four decades earlier, and the increase may be due to influx of the farmers who previously used the private dips.

The oscillating dipping trend observed before 2009 also coincided with implementation of the Kenya Government policies of Ministerial staff rationalization, right-sizing through retrenchment and voluntary early retirement and the liberalizing

delivery of services (Strategy for Performance Improvement in the Public Service, 2001; Poverty Reduction Strategy, 2007). The fluctuation can therefore be attributed to the changes (and possible disruptions) in dip management and regulatory services due to technical staff reduction, inadequate sensitization of farmers, the take-over of technical service facilities by non-technical stakeholders and poor response from the professionals in the private sector. This is supported by observations by Ragwa, (2002); Chema and Gathuma, (2004) and Ngigi, (2005) on skewed uptake of liberalized services by professional personnel in private practice.

The study established that a total of 41,767 cases of tick-borne diseases were recorded during the period. The diseases maintained a constant trend throughout the period, except in 2009 when the cases of anaplasmosis rose relative to decreased levels of ECF. Anaplasmosis and ECF had high incidences compared to babesiosis ((4.55%) and heartwater (0.63%). The high incidence of anaplasmosis (19,939 and 47.74% cases), which is attributed to the blue tick *R.(B). decoloratus*, was a new finding in view of the policy that targets control of the brown ear tick *R. appendiculatus*, which had 19,664 (47.74%) ECF cases. This would call for reconsideration of the blue tick as an important parasite and vector during development of tick control strategies, which include the perennial weekly dipping regime prescribed for the control of brown ear tick (Legal Notices No. 549, 1968: 1995, 1983; 212, 2003; 46, 2013); more so because the two ticks are continually present in the same dairy cattle ecosystem, as observed by Okuthe *et al.*, (2006) and Omore *et al.*, (1999). The low incidences of babesiosis and heartwater during the period concurred with observations by Ngumi *et al.*, (1997) two decades earlier, through surveys on prevalence of haemoparasites in Western Kenya. Whereas there were no records of *Amblyomma variegatum* ticks in Nandi County, low incidences of babesiosis despite abundance of *R. (B) decoloratus*

suggests that the blue ticks were technically with low levels of the causative pathogen, *Babesia bigemina*.

The study found that 13,208 dip-wash samples were submitted for analysis during the period. Out of these, 7,444 (56.36%) indicated that dips had the recommended (normal) dip-wash concentrations, 5,126 (38.81%) had under-strength concentrations, while 638 (4.83%) had over-strength concentrations. Whereas the normal and the over-strength concentrations would destroy susceptible ticks, the presence of under-strength dip-wash was contrary to the prescribed acaricide concentrations recommended by their manufacturers, and enforced by DVS, for efficiency (Maina, 2003).

The recovery of active ticks from the cattle was contrary to observations by Seifert, (2006) that exposure to acaricides would make them restless with detachment within a period of thirty minutes and eight hours. The firm attachment, active motility of recovered ticks and ability to lay eggs that hatched into viable larvae indicated the viability of ticks that survived the dipping. The observations were also contrary to the principles of the study acaricides, which were expected to kill the ticks, or render them unproductive, reduce their capacity to lay eggs or reduce hatchability of laid eggs (Kagaruki, 1996). The persistence of the ticks on dipped cattle could be attributed to use of acaricides at low concentrations, which was observed in 38.81% of the dip-wash samples that were submitted for analysis. This could also be due to an early onset of resistance to the acaricide used, as per FAO, (2004) postulation that first levels of resistance are recognized through failure of a treatment to remove tick burden from cattle. The high mean weights for ticks collected from cattle that were dipped with Chlorfenvinphos (0.12403g) compared to those from Amitraz (0.1182679g) and Alphacypermethrin (0.1135151g) may indicate presence of

populations that were not affected by the dipping but continued to feed. The weights of ticks that survived exposure to Amitraz did not concur with the observations by Kagaruki, (1996) that surviving blue ticks could have a reduction from mean engorged weights of 0.1173g to as low as 0.0177g. The efficacy, too was not as high as the observed 99% elimination rate. This study found that the surviving ticks had mean weights (0.1182679g) that compared fairly with the afore-mentioned engorged adult mean weights of 0.1173g. This implied that the dipping with Amitraz had little impact on the feeding and growth of the ticks. The same conclusion applied for Chlorfenvinphos, contrary to observations by Samish *et al.*, (2004), which noted that the acaricide killed all stages of ticks. The findings were also contrary to those from other workers, who found that acaricides interfered with the feeding of surviving ticks (Seifert, 1986), and on highly susceptible stages in their life cycles (FAO, 1984).

5.2: ADULT IMMERSION TESTS

5.2.1: Efficacies of single acaricides on adult ticks

This study found that the three acaricides, in their standard concentrations, had insignificant effect on *R. (B). decoloratus* adults in 24 hours from exposure (p value = 0.0983). This was contrary to observations by Drummond, (1983), Seifert, (1996), Haque, (2014) that they would kill the ticks within the same period. The low performance could be due to tolerance as a result of emerging, but low level of resistance in the ticks (Baker *et al.*, 1965). Similar cases of low, but temporary resistance levels were observed by Thullner *et al.*, (2007), in *R. (B). microplus* ticks that were exposed alternately to coumaphos and deltamethrin acaricides in Costa Rica. If the same observation applies to the study-ticks, then there is frequent use of different acaricides on cattle in Kapsabet sub-county.

Indeed, the tolerance was temporary, as improved efficacies were seen after 96 hours from exposure, with chlorfenvinphos killing 91.67% of the ticks from the Alphacypermethrin dip category. In the same period of time, Alphacypermethrin had an efficacy of 66.67% on the ticks from Alphacypermethrin dips, and equally efficacious on ticks from Amitraz dips. These findings indicated that the acaricides required longer period to effect appreciable mortalities, contrary to observations by Seifert (2006); Roulston *et al*, 1971; Dudai *et al*, 1987), who observed that 90% of the ticks dropped-off from hosts within eight (8) hours from exposure. The fresh acaricides, in their standard concentrations, did not eliminate the ticks, which contradicted observations by George (2000), that efficacies depended on quantity and quality of active acaricides ingredients accessed by ticks on cattle hosts.

The three acaricides differed on their impact on the tick categories. Significant differences ($\text{Prob} > F = 0.00$) were observed when their mean effects were estimated between the groups of ticks against ticks from the Amitraz category. The multiple comparisons of the efficacy mean between the test acaricides against ticks from amitraz dip, using the Bonferroni post hoc test, identified significant differences between amitraz and chlorfenvinphos acaricides (0.000), alphacypermethrin and amitraz acaricides (0.000), plain water-chlorfenvinphos and plain water-alphacypermethrin acaricides (0.000). Apparently the acaricides were effective against the ticks. This was contrary to findings by Wharton (1983), that resistance commonly occurred within five to ten years from usage of an acaricide. This implied that rotation of acaricides used on cattle could slow down development of resistance in the ticks, as observed by Thullner (2007) and Nolan (1990); and could provide a tentative solution to the tick control problem.

5.2.2: Efficacy of combined acaricides on adult ticks

Observations indicated that combining acaricides increased efficacies relative to their impact in single states. The chlorfenvinphos-amitraz combination had high efficacy on ticks collected from cattle dipped in alphacypermethrin or amitraz, while chlorfenvinphos-alphacypermethrin had improved efficacies on ticks from cattle that used the amitraz dips. These findings concurred with observations by Jongejan *et al* (2015), on combinations of fibronil and permethrin against the dog ticks *Dermacentor reticulatus* and *R. sanguineus*. The amitraz-alphacypermethrin combinations had lower efficacies relative to the first two combinations.

The efficacies of the combinations on adult ticks, were significantly effective only after 96 hours from exposure, as was the case with the single acaricides. These observations were still contrary to findings by Roulston *et al*, 1971; Seifert (2006) that the ticks would die within eight hours after exposure in the first instance.

5.3: LARVAL PACKET TESTS

5.3.1: Assessment of larval susceptibility to single acaricides

The study found that the acaricides had varying efficacies on the larvae from different tick categories. Chlorfenvinphos was 100% effective on larvae from all tick categories, while alphacypermethrin was similarly effective on larvae to ticks from amitraz and alphacypermethrin dips. The lowest efficacy was 50%, observed for amitraz on the ticks from the alphacypermethrin category of ticks.

5.3.2: Assessment of larval susceptibility to combined acaricides

Efficacies that ranged from 95.45% to 100% were observed for the combinations against the larval stages. The mixtures of chlorfenvinphos-alphacypermethrin were 100% effective on larvae from ticks collected from cattle that used the

alphacypermethrin and chlorfenvinphos dips. The same observation was for amitraz-alphacypermethrin mixture on the larvae of ticks from cattle that used chlorfenvinphos dips. This concurred with findings by Van de Merwe, (2005) on efficacies of amitraz-cypermethrin on *R. (B). decoloratus* infesting buffaloes in South Africa. If the same applies for amitraz-alphacypermethrin mixture on the ticks infesting cattle, then their usage will be consistent with those of Haque *et al*, (2014) on ability of acaricides to break the life cycles of ticks. Results from this study concurred with observations by Jongejan *et al*, (2015), Dumont *et al*, (2015), Daemon, (2015) that combinations of some acaricides improved their efficacies against a variety of ticks. Research by Jun-Hyung and Murray (2015) had found that combinations had enhanced penetration abilities, while those from Kunkle *et al* (2012) and Jongejan *et al*, (2015) found enhanced toxicities.

5.4: SUMMARY ON EFFICACIES OF ACARICIDES ON ADULT AND LARVAE

The single acaricides were effective on the adult stages of *R. (B). decoloratus* ticks after 96 hours from exposure, but effective on the larvae in 24 hours. The acaricides were highly efficacious on larvae than adult ticks.

Combining the acaricides produced efficacies ranging from 0 to 100% on adults, with most of the efficacies falling below 70%, compared to ranges of 97.7% to 100% on larval stages.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1: CONCLUSIONS

- 1) Tick control services in Nandi County were mainly offered through an average of 286 communally-managed plunge dips, with another 45 dips being operated privately by individual farmers. The tick control service delivery in the County was not assessed between the years 2000 and 2012. Assessment on performance of the services, more so during the initial phases of Structural Adjustment Programmes when the dips were hurriedly off-loaded from Government support to inadequately-sensitized users, would have been necessary.
- 2) Participatory decision-making from sensitized stakeholders in the livestock industry were not employed to facilitate sustainable uptake of the liberalized tick control services. The farmers registered 266,178 \pm 30,63 cattle for dipping, which was lower than the population of 309,039 cattle that established in Census to be in the County. At least 39% of the dip-wash used on the cattle had lower acaricide concentrations than recommended. Anaplasmosis and ECF were the most prevalent tick-borne diseases in the County.
- 3) Chlorfenvinphos, amitraz and alphacypermethrin acaricides, used for tick control in the County, were still efficacious against adult and larval stages of blue ticks. The standard acaricide dilutions required at least 96 hours to effect mortalities on adult ticks, but only 24 hours on the larval stages. Binary combinations of the acaricides had higher efficacies on the adult and larval stages of the ticks than in their single states. The combinations had higher efficacies on larvae within 24 hours, unlike adults that still required 96 hours.

The dreaded onset of widespread tick resistance to the acaricides appeared to be minimal, and can still be delayed through acaricide rotation.

- 4) Some of the ticks that were recovered from dipped cattle were viable, and laid large masses of eggs that had virtually 100% hatchability to produce active larvae. This showed possibilities of resistant tick populations to the acaricides, as used in routine dipping.

6.2: RECOMMENDATION FOR POLICY AND PRACTICE

1. Farmers should use acaricides judiciously for effective cattle dipping and minimization of tick resistance. Stakeholder participation in tick control projects and programmes should be a mobilization tool for ownership of change processes. The stakeholders should clearly know their roles in all stages of tick control.
2. Comprehensive assessment on the performance of tick control programmes and monitoring of tick population dynamics, including livestock movement, should be a routine activity in the County and country.
3. The life cycles of prevalent tick species in different zones should be factored into tick control programmes.
4. Logistics of how to alternate usage of acaricides within a vicinity should be developed, with review of the zoning programme, to allow for periodic rotation of cattle dipping as means to enhance destruction of persistent ticks.
5. There is need to develop a strategy on dipping intervals for the *R. (B) decoloratus* and *R. appendiculatus* ticks in the ecosystem on account of their relative numbers of hosts.
6. There is need to quantify synergies in acaricide combinations for use in control of *R. (B) decoloratus*, and other ticks in Kenya.

7. Surveillance on tick populations and monitoring of their resistance to acaricides should be regularly maintained.

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