ETIOLOGY, PREVALENCE, RISK FACTORS AND PATHOLOGICAL LESIONS ASSOCIATED WITH CANINE CUTANEOUS MYIASIS IN KITUI COUNTY, KENYA

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This thesis is my original work and has not been presented for the award of a degree in any other University or institution of higher learning.

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DEDICATION

To my loving wife (Grace Malia), my daughter (Elizabeth Mbuli), my brothers, sisters and to all my friends. We finally made it!

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LIST OF ABBREVIATIONS AND ACRONYMS

>	Greater than
<	Less than
b.w.t	Body weight
CCM	Canine cutaneous myiasis
Cm	Centimeters
DPX	Dibutylphthalate Polystyrene Xylene
GPS	Global Positioning System
H &E	Hematoxylin and Eosin
HCL	Hydrochloride
HCM	Human cutaneous myiasis
I.m	Intramuscularly
Kg	Kilogram
Max	Maximum
Mg	Milligram
Min	Minimum
Mls	Millilitres
Mm	Millimeters
Spp	Species
TVT	Transmissible venereal tumor
UoN	University of Nairobi

ABSTRACT

A cross-sectional study of canine cutaneous myiasis (CCM) was conducted from March to August, 2021 in eight sub-counties of Kitui County, Kenya. The aim of the study was to determine the etiology, prevalence, risk factors and pathological lesions associated with canine cutaneous myiasis in dogs. Face to face questionnaire interviews were conducted for assessment of risk factors that determine the occurrence of canine cutaneous myiasis in the study area. A total of 400 dogs were physically examined for the presence of skin lesions characteristic of canine cutaneous myiasis. At least two larvae were collected from each dog by application of digital pressure at the base of the lesions. The fly larvae causing CCM were preserved in 70% ethyl alcohol, transported to laboratory where they were processed, characterized and identified. From 400 dogs sampled, 180 (45%) had furuncular skin lesions due to third stage larvae of myiasis causing flies. These furuncles were concentrated on the ventral parts of the body of the dogs. Two cases of cutaneous myiasis in goats were also encountered during the study. Intensity of infestation was 434 and 6 dipteran maggots from dogs and goats, respectively. All dipteran larvae samples (440) were identified as *Cordylobia anthropophaga* fly larvae (100%). Freshly harvested *C. anthropophaga* larvae were cultured for four weeks and adult "tumbu" flies hatched, captured and identified. Analysis of questionnaires using chi-square revealed that there was a statistically significant association among different dog breeds, housing structures, their hygiene measures and environmental hygiene ($p<0.05$) and myiasis. However, there was no statistically significant differences in the prevalence of canine cutaneous myiasis among different dog age and sex groups ($p>0.05$). Complete canine necropsy examination was conducted in four dogs purchased with severe canine cutaneous myiasis infestation from Kitui County. Grossly there were multifocal furuncular skin lesions extending throughout the thickness of the skin epidermis to the dermis.

The skin lesions were characterized by thick, firm consistency, cutaneous edema and congestion. Some of these had live maggots. The underlying skeletal muscles were bright red due to hyperemia. There was regional lymphadenopathy in affected body regions. Microscopically, there was disruption, discontinuity and desquamation of the stratified squamous epithelium of stratum corneum of the skin which were the entry points for the *C. anthropophaga* larvae into the dogs` skin. There were circular cavities surrounded by a fibrous capsule, mixed inflammatory cells infiltrate with predominant eosinophils. Underlying skeletal muscles had coagulative necrosis, proliferation of fibroblasts and deposition of eosinophilic collagen fibres and infiltration of mixed inflammatory cells. During necropsy, various ectoparasites and endoparasites were recovered and included ticks, fleas, tapeworms and nematodes, an indication of poor dog management practices in the area.

In conclusion, *C. anthropophaga* larvae of tumbu fly were the etiological agent associated with canine cutaneous myiasis, that was predisposed by poor housing and hygiene and had high occurrence and caused furuncular lesions in animals in Kitui County, Kenya. More studies on seasonal prevalence, distribution in various counties, etiological survival limits, public health importance and control strategies of CCM in the country are recommended.

CHAPTER ONE

1.0 INTRODUCTION

Myiasis refers to the infestation of the living tissues of human and other animals with dipterous larvae for a particular duration whereby the larva feed on the host's liquid body substances, ingested food or dead or living tissue (Zumpt, 1965). After infestation of the host animal, diptera larvae complete part of their normal development in or on the vertebrate body (Maria *et al.*, 2006).

Myiasis is more often found in domestic animals in the tropics globally especially in developing countries where it is associated with poor animal welfare (Yanuartono *et al.*, 2019). It is mainly predisposed by low levels of animal hygiene, cage and environment hygiene (Yanuartono *et al.*, 2019) but there is a considerable research gap on the specific predisposing factors for canine cutaneous myiasis in Kenya. The condition is fairly common but underestimated in many rural areas (Faramarzi *et al.*, 2009) despite its great medical and veterinary importance (Obanda *et al.*, 2013). Myiasis causing flies are attracted by skin injuries and excretory products such as urine and feces in the animals` environment. Female flies deposit eggs in the skin wounds or on sleeping areas especially beddings, sand or straws (Ogo *et al.*, 2012).

Fly eggs hatch and resulting larvae penetrate the animal skin after coming into direct contact from the environment or beddings where it feeds on dead or living tissues and body fluids in the body of the host animal (Stevens and Wall, 2001). Growth rate and development of the fly larvae from L_1 to L_3 stage (first instar to third instar) and the pupal stage is determined by the diptera fly species, environmental temperature and to a lesser extent by humidity (Stevens and Wall, 2001). Factors which determine the occurrence of clinical myiasis in both humans

and animals include high ambient temperature, humidity, rainfall intensity and host susceptibility (Urquhart *et al.*, 1996).

Cutaneous myiasis (CM) can be classified into three categories on the basis of clinical manifestation of the disease in the cutaneous system: furuncular, creeping and wound myiasis (Spradbery, 2002; Faramarzi *et al*., 2009). Furuncular myiasis is caused by dipterous larvae such as *Cuterebra* species, *Dermatobia* spp., *Wohfartia vigil* and *Cordylobia* spp. Oliva *et al.* (2020) reported human furuncular myiasis due to *Cordylobia anthropophaga* which was diagnosed in Bergamo in Italy in an Italian tourist who had visited Watamu in Kenya but there is no published data on the etiological agent(s) for canine furuncular myiasis in Kenya. Creeping myiasis in man is caused by the genera *Gasterophilus* and *Hypoderma* (Spradbery, 2002; Faramarzi *et al*., 2009). Wound myiasis is caused by diptera flies such as *Chrysomya bezziana, Cochliomyia hominivorax, Lucilia sericata* and *L. cuprina* among others (Spradbery, 2002; Faramarzi *et al*., 2009).

In tropical regions such as Africa and subtropical regions such as South America non migratory cutaneous myiasis of both livestock and man are usually caused by *Cordylobia* spp. such as *Cordylobia anthropophaga, C. rodhaini, C. ruandae* and *Dermatobia hominis*, respectively (Olumide, 1994; Tamir *et al.*, 2003; Adam *et al.*, 2006; Johnson *et al.*, 2016). McGraw and Turiansky (2008) reported that *C. anthropophaga* is the principal etiological agent of cutaneous canine myiasis (CCM) because both man, dogs and rodents serve as the reservoir hosts for the larvae but there is no published data on the etiological agent(s) of canine cutaneous myiasis in Kenya.

Cases of human myiasis have been reported in some African countries including Nigeria (Ogo *et al.*, 2009) and Sudan (Adam *et al.*, 2006). Travel to and from tropical countries has led to a rise in clinical cases of human myiasis in non-endemic areas (Tamir *et al.*, 2003; Dehecq *et al.*, 2005; Robbins *et al.*, 2010; Palmieri *et al.*, 2013).

Kitui County has semi-arid and arid climatic zones with temperatures ranging between 14°C and 32°C (Anonymous, 2018). This temperature range was previously reported as a standard environmental condition for breeding and spread of dipterous larvae associated with CCM (Johnson *et al.*, 2016). However, this has not been investigated.

1.1 Problem statement

In Kenya a case of myiasis in wild game eland was reported by Obanda *et al.* (2013) however, there are no published work on the prevalence of cutaneous myiasis in both humans and livestock in Kenya. Despite CCM being a zoonotic disease and its associated public health importance, publications on the etiological agent (s), prevalence, lesions and their distribution on the animal body and risk factors associated with the occurrence of this condition in Kitui County and Kenya are limited.

1.2 Study justification

Cases of canine cutaneous myiasis (CCM) have been reported in Africa due to *Cordylobia anthropophaga* maggots in dogs (Ogo *et al.*,2009; Abebe, 2017). Mugachia (2018) reported in a newspaper the observation of canine cutaneous myiasis in Kitui County, Kenya. Cases of human cutaneous myiasis due to *Cordylobia anthropophaga* acquired in Kenya but diagnosed elsewhere have been reported (Oliva *et al.*, 2020), but published reports on public health significance of canine cutaneous myiasis are unavailable in Kenya.

There is a need to determine the etiology, prevalence, risk factors and pathological lesions associated with canine cutaneous myiasis in dogs in Kitui county.

1.3 Objectives

1.3.1 General objective

To determine the etiology, prevalence, risk factors and pathological lesions associated with canine cutaneous myiasis in Kitui County, Kenya.

1.3.2 Specific objectives were to:

1. Determine risk factors associated with occurrence of canine cutaneous myiasis in Kitui County, Kenya
2. Determine prevalence; characterize and identify etiological agent(s) of canine cutaneous myiasis in Kitui County, Kenya
3. Determine gross and microscopic lesions associated with canine cutaneous myiasis in Kitui County, Kenya

1.4 Research hypotheses

1. There are no risk factors associated with canine cutaneous myiasis in Kitui County
2. Canine cutaneous myiasis is not prevalent in Kitui County and *Cordylobia anthropophaga* maggots are not the causative agent of canine cutaneous myiasis in Kitui County
3. There are no gross and microscopic lesions associated with canine cutaneous myiasis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of myiasis

There are two ways for classification of myiasis, namely, biological or anatomical methods. Biological (parasitological) classification is based on the host-parasite relationship while anatomic one is based on the affected part of the host (Kenawy, 2019).

2.2 Biological classification

This classification includes: -

2.2.1 Obligatory myiasis

This is also referred to as specific myiasis. The diptera maggots live on live host to complete part of their development. The diptera flies infest and deposit their eggs in various body organs or tissues of live host animal. These include *Wohlfahrtia magnifica* and *W. vigil, Oestrus ovis, Hypoderma bovis, Gasterophilus intestinalis, Cochliomyia* spp, *Chrysomya bezzi*ana and *Dermatobia hominis* (Kenawy, 2019).

2.2.2 Facultative myiasis

In facultative or opportunistic myiasis the diptera larvae develop on either living or decayed organic matter. Diptera flies attack only dirty infected wounds in a live host whereby they are attracted by the foul odor but don't attack clean surgical wounds or intact skin. Kenawy (2019) reported that the development of diptera larvae causing facultative myiasis occurs under three levels of development. Primary strike whereby the diptera larvae are free living and able to initiate myiasis. Secondary strike whereby the diptera larvae are unable to initiate myiasis hence they parasitize as secondary species. Tertiary strike whereby these are diptera fly larvae which parasitize the live host prior to its death. Examples of facultative

(opportunistic) myiasis causing flies include: *Calliphora* spp. (Blue bottle flies) and *Lucilia* spp. (Green bottle flies), *Sarcophaga* spp. (Flesh flies), *Piophila casei* (Cheese skipper) and *Tubifera tenax* (Rat-tailed maggot) (Kenawy, 2019).

2.2.3 Pseudo myiasis

Pseudo (accidental) myiasis are free living nonparasitic diptera larvae that are accidentally swallowed (Zumpt, 1965). Examples of such flies include the family Muscidae (Nagakura *et al.,* 1991). It is not clear which biological myiasis occur in Kitui County.

2.3 Anatomical classification

2.3.1 Sanguinivorous myiasis

This describes blood sucking diptera larvae which infest the skin of host animal and are able to initiate obligatory myiasis. They include *Chrysomia bezziana* and *Auchmeromyia luteola* (Family Calliphoridae), *Dermatobia hominis* (Family Oestridae; Zumpt, 1965).

2.3.2 Cutaneous myiasis

The obligatory myiasis dipterous larvae feed on the epidermal and dermis layers of the skin causing wounds. These wounds or traumatic wounds either caused by insects or other agents attract obligatory or facultative dipterous flies which cause myiasis (Gour *et al.,* 2017)**.** Dipterous flies of the families Calliphoridae and Sarcophagidae produce both dermal and traumatic (wound) myiasis (Gour *et al.,* 2017).

2.3.3 Furuncular myiasis

This refers to cutaneous myiasis which occurs after the **penetration of healthy skin by dipterous larvae causing cutaneous furuncular lesions**. The furuncular lesions clinically presents as nodular lesions with a central pore that exudates pus or serous fluid after diptera larval infestation. The erythematous lesion, pruritus, movement sensation, hyperpigmentation,

fluid leak from nodes and night pain are reported as symptoms of furuncular myiasis (Mahal and Sperling, 2012). The number of furuncular lesions and their distribution patterns on the host animals are dependent on diptera fly species. These include: *Cordylobia anthropophaga*, *Dermatobia hominis, Wohlfahrtia vigil, W. magnifica,* and *Cuterebra* spp. (Robbins and Khachemoune, 2010). Previous reports by Mugachia (2018) on the occurrence of furuncular myiasis in dogs in Kitui County necessitate further investigations on the etiological agent(s), prevalence, lesions and their distribution on the animal body and the risk factors associated with the occurrence of this condition in Kitui County.

2.3.4 Migratory myiasis

Migratory or creeping myiasis occurs when maggots start to migrate for a distance in the host skin and burrow in it causing obvious migratory lesions. This migration produces ulcerated epithelium and causes inflammation of the dermis layer. The larvae are then diagnosed in the fibrous cystic sinus tract in dermis layer (Gour *et al.,* 2017). *Hypoderma bovis* (Cattle bot fly) and *Gasterophilus intestinalis* (Horse bot fly) are common agents of migratory or creeping myiasis. Humans could be accidentally infested by both cattle and horse bot flies but the larvae do not complete their life cycle in human skin (Royce *et al*., 1999).

2.3.5 Cavity myiasis

This refers to the infestation of body cavities by larvae of diptera flies (Francesconia and Lupi, 2012).

2.3.6 Ocular myiasis

Ocular or ophthalmic myiasis occurs due to internal, external or intraorbital infestation of host eyes by dipterous larvae. Ocular myiasis clinically presents as red eyes, pain, and blindness (Anane and Hssine, 2010). Ocular myiasis is caused by larvae of diptera flies such as *C. bezziana*, *D. hominis*, *Lucilia* spp. and *Cuterebra* spp. (Francesconia and Lupi, 2012).

2.3.7 Oral myiasis

This is commonly associated with oral trauma, poor oral hygiene and gingival diseases (Hassona *et al*., 2014). Oral myiasis clinically presents as swelling of mouth, cheilitis and gingivitis. It is commonly caused by diptera fly larvae such as *Chrysomya bezziana*, *Cochliomyia hominivorax, Musca domestica, Wohlfahrtia magnifica* and *Calliphora vicina* (Hassona *et al*., 2014).

2.3.8 Aural myiasis

This is the infestation of the ear canal by dipterous fly larvae. Female flies oviposit eggs around the aural cavity (Uzun *et al.,* 2004). The clinical presentation of aural myiasis include itching sensation, inflammation of auditory canal, aural pain to otorrhea and bleeding. The most common etiological agents of aural myiasis include *C. bezziana, C. megacephala, C. hominivorax* and *W. magnifica* (Francesconia and Lupi, 2012).

2.3.9 Nasal myiasis

This occurs when female diptera flies deposit their eggs or first stage larvae in the nasal cavity while the host animal is sleeping (Francesconia and Lupi, 2012). Development of larvae in the nasal-sinus cavities can cause severe clinical signs including nasal pain, mucoid nasal discharges, hemoptysis and breathing difficulties, which together with the annoyance caused by the adult flies, may lead to significantly reduced animal production and economic losses (Tsang and Lee, 2009; Sotiraki *et al.,* 2012). The common diptera flies which cause nasal myiasis include: *C. bezziana, C. hominivorax, Oestrus ovis, Lucilia sericata, W. magnifica, Drosophila melanogaster* and *Cephalopina titillator* (Camel bot fly) (Francesconia and Lupi, 2012).

2.3.10 Intestinal myiasis

Intestinal or enteric myiasis is more common in animals and diptera fly larvae are harbored in the alimentary tract of the host animals from pharynx to the anus. Intestinal myiasis is usually an accidental phenomenon, which occurs due to the ingestion of eggs or larvae present in food (Sehgal *et al.*, 2002). Usually the patient is asymptomatic and the larvae are excreted harmlessly in the feces (Sehgal *et al.*, 2002). Clinical presentation varies from asymptomatic cases to nausea, vomiting, abdominal pain, rectal bleeding and rectal prolapse (Karabiber *et al.*, 2010). The most common causative agent of intestinal myiasis is *Gasterophilus intestinalis* (Singh and Singh, 2015).

2.3.11 Urogenital myiasis

This is the infestation of urogenital area of the host by dipterous larvae. It is usually associated with poor general health and hygiene (Zaidi *et al.*, 2016). The most common symptoms are lumbar pain, ureteric obstruction, and dysuria. The causing flies are *Wohlfahrtia magnifica, Sarcophaga haemorrhoidalis, S. carnaria* and *Fannia canicularis* (Francesconia and Lupi, 2012).

2.3.12 Cerebral myiasis

This is rare, fatal, and associated with low survival rates (Terterov *et al.*, 2010). Cerebral myiasis is caused by diptera fly larvae of *Hypoderma bovis* and *H. lineatum* (Francesconia and Lupi, 2012) and *Dermatobia hominis* (Rossi and Zucoloto, 1973). There is need to investigate the presence of and type of anatomic myiasis in Kitui County.

2.4 Clinical signs associated with cutaneous myiasis

The typical clinical presentation of CCM lesions includes multiple erythematous, nodular (furunculoid) lesions which ooze serous fluid or pus (Henok, 2017). The nodular lesion

mostly harbors one dipterous larvae but multifocal coalescent cutaneous nodular lesions can also be found on affected body sites (Henok, 2017). In humans the symptoms of myiasis include painful nodular swellings about 1cm in diameter with a small central opening where the larvae put their spiracles for breathing (Soulsby, 1986) with surrounding indurated erythema and fever (Ogo *et al*., 2012).

2.5 Diagnosis of myiasis

Presumptive diagnosis of cutaneous myiasis is concluded from clinical presentation of multiple erythematous furunculoid serous or suppurative lesions with fly larvae on the skin. Confirmatory diagnosis is based on manual extraction and laboratory morphological characterization and identification of the diptera flies' larvae. This is based on the morphology of anterior hooks, number of body segments, presence, arrangement and number of cuticular spines on the body segments and posterior spiracular slits located in the posterior spiracular plates (Soulsby, 1986). Maggot culture and identification of adult diptera fly species is confirmatory (Ogo *et al.*, 2012).

2.6 Morphology and lifecycle of *Cordylobia anthropophaga*

Cordylobia anthropophaga (tumbu fly) is a stout, compactly built fly about 9.5mm long (Soulsby, 1986). The adult tumbu fly has a brown color characterized by bluish-grey thoracic and dark-grey posterior abdominal patches (Soulsby, 1986). The larva is approximately 12mm long. On the anterior end it has a pair of oral hooks with a number of minute cuticular spines which are sparsely distributed on the thoracic and abdominal body segments. The posterior end has two spiracular plates each with three spiracular slits (Soulsby, 1986).

The adult female fly deposits some 500 eggs in the sleeping places of man and various animals on the ground or on the straw and on clothing that smells of perspiration (Soulsby, 1986).

The eggs hatch after 2 to 4 days and the larvae penetrate into the skin of the host animal where they mature in 8-15 days (Soulsby, 1986). The mature larvae then leave the host animal and pupates in the ground for 3-4 weeks before the adult fly emerges (Soulsby, 1986).

2.7 Morphology and lifecycle of *Wohlfahrtia* species

The adult flies are medium sized to large with thick set of a light or dark grey color. They have a plumose arista to about its middle and bare in the distal portion (Soulsby, 1986). The thorax often has three longitudinal dark stripes and the dorsum of the abdomen has dark spots or is chequered dark and grey (Soulsby, 1986).

The larvae are large, robust and over 15 mm long with very stout spines. The larva don`t have a definite sclerotized head capsule. Body is smooth or with short spines, but no long lateral processes. Posterior spiracles not on peg-like tubercles. The body is swollen or tapered posteriorly but never extended into a tail-like process. Peritreme absent or if present without three distinct slits. Posterior spiracles have three distinct slits with spiracular slits straight and sunken in deep cavity (Dodge, 1966).

Species of this genus are larviparous. *Wohlfahrtia magnifica* and *Wohlfahrtia vigil* deposits its larvae in the external ear of man or in the sores around the eyes or elsewhere on the bodies of man and other animals in which the larvae develop (Soulsby, 1986).

2.8 Morphology and lifecycle of *Dermatobia hominis*

The adult female flies are about 12 mm long. The thorax is dark blue with a greyish bloom, the abdomen is short and broad and has a brilliant blue color (Soulsby, 1986). Mature larvae

are about 25 mm long (Soulsby, 1986). Mouth-hooks are prominent but not enclosed in a definite head capsule (Hall and Smith, 1993). The thoracic and abdominal body segments have strong grub like spines. Posterior spiracles are sunk in a deep cavity, which closes over and conceals them (Hall and Smith, 1993). The posterior spiracles have three straight or curved sinuous slits (Hall and Smith, 1993).

The adult flies do not feed and nourishment is derived from the food stores accumulated during the larval period. When the adult fly is ready to oviposit, it captures a mosquito or blood sucking fly and glues a batch of eggs on the abdomen of the captive fly. When the transport fly alights on a warm blooded host the larvae of *Dermatobia hominis* hatch from the eggs and penetrate the skin of the host through the skin puncture made by the blood sucking fly. About six days are required for the eggs to reach the stage of hatching, but this occurs only when the carrier fly settles on a suitable animal to feed (Souslby, 1986).

As the larvae grows it produces a swelling which has a central opening through which it breathes. Development in the host requires 5-10 weeks, after which the larvae escapes and pupates in the ground for 5-10 weeks before the adult fly emerges (Souslby, 1986).

2.9 Morphology and lifecycle of *Cuterebra emasculator*

They are large flies >20 mm long. Bodies are bee-like with vestigial mouth parts (Soulsby, 1986). Mature larvae are stout measuring up to 25 mm in length. Mature larvae have well developed anterior mouth hooks. The thoracic and abdominal body segments have strong evenly distributed cuticular spines. Mature larvae are parasitic under the skin of rodents (Soulsby, 1986). Mouth hooks are well developed and body with evenly distributed cuticular spines (Hall and Smith, 1993). Posterior spiracles are located on posterior face of terminal segment. Posterior spiracles have large number of small pores or many short intertwined serpentine slits arranged in three groups on each spiracular plate (Hall and Smith, 1993).

The adult flies oviposit near the entrance of the burrows of rabbits, mice and chip-monks. Larvae hatch at intervals and penetrate the skin of the above hosts producing cyst like subcutaneous lesions in which the larvae mature. Mature larvae are produced in about one month, at which stage they are dark in color and covered with bands of spines. Youngest larvae are lighter in color. The larvae leave the host to pupate in the soil. *Cuterebra emasculator* frequently parasitize the scrotum destroying the testes and causing parasitic castration (Soulsby, 1986).

2.10 Management of myiasis

In man application of digital pressure on either side of the furunculoid lesion help in expulsion of the fly larvae and prescription of broad spectrum antibiotics are recommended for signs of bacterial infection (Caissie *et al.,* 2008). In dogs, subcutaneous injection with Ivermectin (0.4-0.6mg/kg body weight) is the definitive treatment of CCM**.**

2.11 Prevention and control of myiasis

This mainly relies on the application of stringent hygienic measures in the dog kennels and prompt treatment of all clinical cases of CCM with recommended insecticides. Regular cleaning and removal of dog feces and decomposing waste materials which might serve as the breeding sites for the diptera flies from the dog kennels should be done regularly (Abebe, 2017). Cleanliness and regular disinfection of sleeping places is important (Soulsby, 1986). In valuable animals such as Angora rabbits which are frequently affected by cutaneous myiasis can be protected by keeping the diptera flies off with a gauze wire (Soulsby, 1986).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in all the eight sub-counties (Kitui central, Kitui west, Kitui east, Kitui rural, Kitui south, Mwingi north, Mwingi central, Mwingi west) of Kitui County (Figure 3.1). The county is located approximately 160Km from Nairobi and in the former Eastern Province. It lies between latitudes 0°10 and 3°0 south and longitudes 37°50 and 39°0 and altitude ranging 400-1800m above sea level (Anonymous, 2018). Selection of the study area was based on the fact that CCM is endemic in Kitui County as reported in the Daily nation media (Mugachia, 2018).

The county has arid and semi-arid climatic zones with the majority of the region having arid climate (Anonymous, 2018). It experiences a bimodal rain pattern, with a high variability in annual rainfall, ranging between 500-1050mm.The topography of the landscape influences the amount of rainfall received (Anonymous, 2015). The highland areas of Mumoni Hills to the north, Kitui Central, Mutitu and Endau hills receive 500-1050mm per year, Migwani and Mutha hills receive between 500-760mm of rainfall per year, while the drier lowlands stretching from the north (Tseikuru, Kyuso, Mwingi, Ngomeni, Nguni and Nuu), through the Yatta plateau, the eastern areas (Mutito and Mwitika), and southern areas (Mutomo and Ikutha), receive less than 500mm. The short rain in October to December are more reliable and are the county's principal productive season. The long rain between March and May rains are usually unreliable (Anonymous, 2015). The lowest annual average temperature in the county is 14°C and the highest annual average temperature is 32°C (Anonymous, 2018).

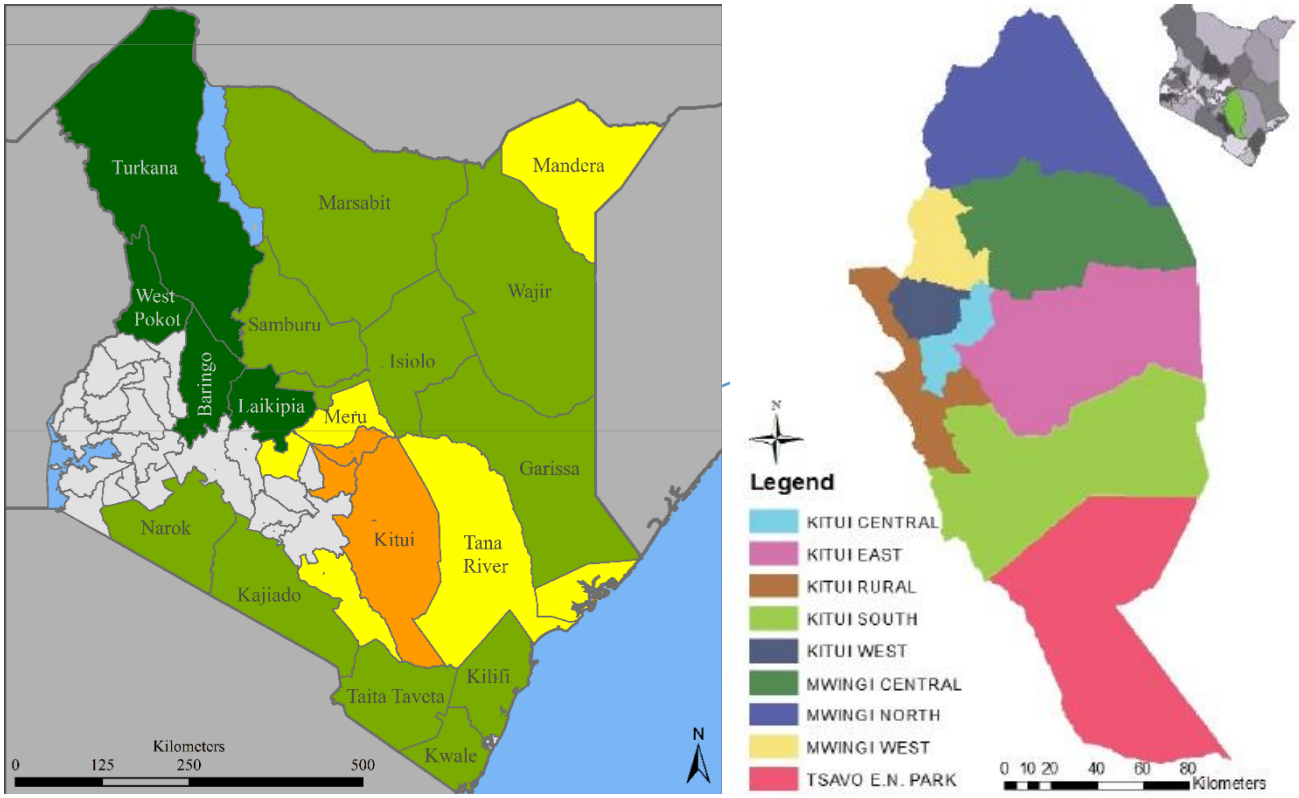

Figure 3.1: Map of Kenya showing Kitui County (in brown) and its eight sub-counties (Klisch and Atzberger, 2016)

3.2 Ethical clearance and consent of dog owners

Ethical approval and clearance to undertake this study was sought and obtained from the Faculty of Veterinary Medicine, University of Nairobi, Faculty Biosafety, Animal use and Ethics committee**; REF: FVMBAUEC/2021/303** (appendix 1.0). Individual verbal consent was sought from individual dog owners in the randomly selected households in Kitui County to allow for physical examination prior to sampling of the infested dogs and participation of the dog owners in the questionnaire interviews.

3.3 Study design and target population

This was a cross-sectional study carried out from March to August, 2021 in Kitui County.

The target animal population were dogs of different breeds, age and sex from various households in the county to ensure systematic random selection of sampled animals. All the eight sub-counties of Kitui County were included in this study. During sampling, the selection of the specific administrative wards was based on a list of ongoing anti-rabies vaccination schedule with concurrent treatment of dogs infested with cutaneous myiasis during vaccination by Kitui County Directorate of Veterinary services. The last ward in each sub-county in the vaccination schedule was selected for sampling. Systematic random selection of the villages and specific households was based on a list of villages and households at the Chief's office, with households selected at an interval of five households.

3.4 Dog sample size

A 50% prevalence was used due to lack of previous studies in Kitui County on CCM, a 5% precision and a 95% confidence interval was used to determine the sample size using the formulae described by Thrusfield (2005).

$$N = \frac{1.962[p\,expected(1-pexpected)]}{D^2}$$

Where the abbreviations denote:

N - Total sample size; p-expected - Expected prevalence; D^2 - despaired absolute precision.

$$N = \frac{1.962 \times 0.5(1-0.5)}{0.052}$$

N=384 dogs

Four hundred dogs from 248 households were examined and sampled and included in this study; a maximum of three dogs of different ages or breeds and either sex was examined and sampled per household.

3.5 Questionnaire administration and evaluation of risk factors associated with the occurrence of canine cutaneous myiasis in Kitui County

Semi-structured questionnaires on biodata of dog owners, dogs, common dog diseases; knowledge, attitude and practices of the residents on CCM was included (appendix 2.0). The questionnaires were administered through face to face interviews with the dog owners in the study area. During sampling, names and contacts of the dog owners were not recorded for confidentiality. Smart phones (Tecno Camon16) was used to take pictures of the infested dogs. Latitude and longitudes app was installed from google play store to help in reading of GPS coordinates of the sampled households before entry into the questionnaires. Eighty (80) questionnaire interviews were conducted from 248 households and responses recorded in hard copy questionnaires.

3.6. Evaluation of prevalence of canine cutaneous myiasis in Kitui County

3.6.1 Selection of sampled dogs

A maximum of three dogs of different ages or breeds and either sex were examined and sampled at each selected households whose owners were available during the household visits and willing to have their dogs examined and sampled in Kitui County. The dogs were classified into various categories depending on the purpose for which they were kept such as pets, security, pet and security, hunting, hunting and security and breeding dogs. Dogs aged

< 6 months were considered as puppies, 6 - 12 months as young adults and > 12 months as adult dogs (Thrusfield, 2005).

3.6.2 Restraint, physical examination and sampling of dogs

A total of 400 dogs were examined and those infested with CCM were sampled from all sub-counties of Kitui county. Sixty dogs were examined in each of the sub counties of Kitui central, Kitui west, Kitui rural and Kitui east whereas, only forty dogs were examined from each of the four sub-counties of Kitui south, Mwingi central, Mwingi north and Mwingi west (Table 3.1). During sampling particulars of the dogs such as sex, age and breed were recorded.

Table 3.1: Number of dogs examined for myiasis lesions in Kitui County

Sub county	**Number of dogs examined**
Kitui central	60
Mwingi north	40
Kitui south	40
Kitui rural	60
Mwingi central	40
Mwingi west	40
Kitui west	60
Kitui east	60
Total	**400**

Puppies and young adults were restrained using small sized dog muzzles by the dog owners for physical examination of their cutaneous system. Young and adult dogs were restrained using a leash and medium and large sized dog muzzles depending on the size of the dogs for physical examination of their skin for the presence of furunculoid lesions caused by CCM.

At least two larvae were harvested by application of gentle digital pressure at the base of the furunculoid lesions to express the dipterous larvae (Figure 3.2). The harvested larvae were immediately preserved in universal bottles containing approximately 30mls of 70% ethyl alcohol (ethanol). The preserved larvae were transported in cool boxes to Parasitology laboratory, Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kenya for characterization and identification of the etiological agent(s) of CCM in Kitui County. After sampling the dogs infested with canine cutaneous myiasis were treated

with Supermec®(Ivermectin) at a dosage rate of 0.4-0.6mg/kg b.w.t subcutaneous route. Dog owners of dogs infested with other conditions were advised to seek Veterinary assistance from the nearest Kitui County Subcounty Veterinary office.

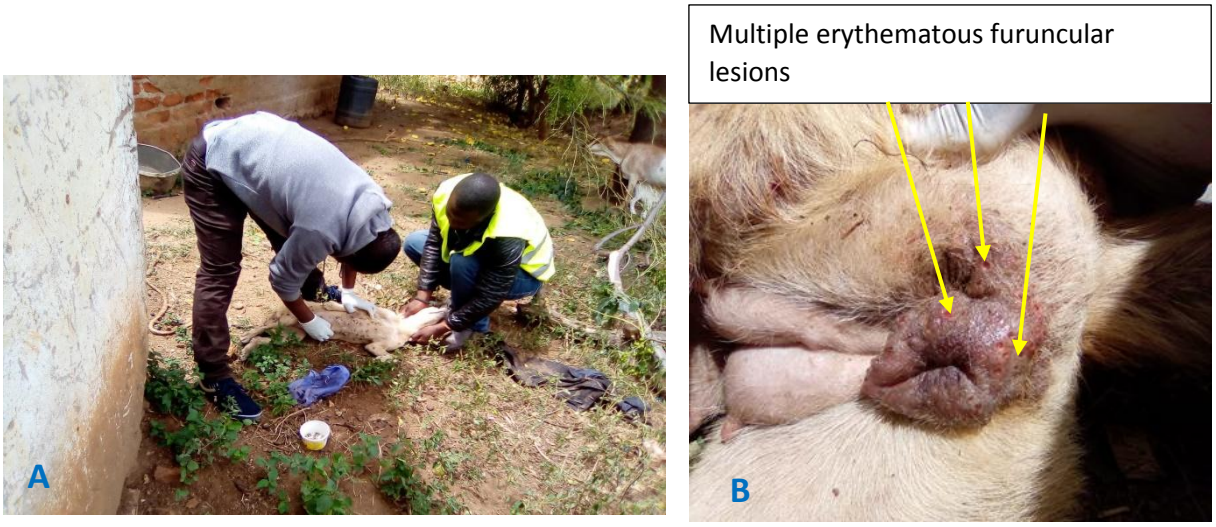

Figure 3.2: Physical restraint and collection of dipterous larvae in a 4 years old bitch (A). Multiple erythematous furunculoid lesions with central pores affecting vulva of the bitch (B)

3.7 Restraint, physical examination and sampling of other animals

Two cases of cutaneous myiasis in goats were encountered in Kitui County during sampling. Physical restraint was used on the infested goats. Maggots were collected from cutaneous furunculoid lesions on the flanks of a goat kid and other larvae were collected from similar lesions on a billy goat scrotal skin. These larvae were squeezed out by application of digital pressure at the base of the furuncles. They were preserved, characterized and identified as stated above.

3.8 Necropsy examination and sample collection from dogs with myiasis

3.8.1 Sedation and euthanasia of sampled dogs

Four dogs heavily infested with CCM were purchased from Changwithya East ward (Nzunguni, Umuu, Kathungu and Kyulu villages), Kitui Central Sub-county, Kitui County

and transported to the Department of Veterinary Pathology, Microbiology and Parasitology for euthanasia, necropsy examination and histopathological sampling. They were sedated using Xylazine HCl at a dosage rate of 1.1mg/kg b.w.t i.m and then put into general anesthesia using a combination of Xylazine HCl and Ketamine 5% at a dosage rate of 1.1mg/kg b.w.t and 2.2. mg/kg b.w.t, respectively. The dogs were then euthanized by intracardiac injection of 30 mls of Lignocaine HCL 2%.

3.8.2 Necropsy technique and sample collection

Canine complete necropsy technique was carried out as described by Mcdonough *et al.* (2017). On external examination, any ectoparasites were immobilized by use of cold water to wet the dog carcasses and collected by use of thumb forceps and preserved in 70% ethyl alcohol.

The location, distribution, size (length and diameter of the furuncular central pore), extent, shape and color of the furuncular skin lesions in the dog carcasses were noted, measured and recorded. The underlying tissues (cutaneous and muscles) were examined for gross changes (Caswell and Platner, 2012).

The skin and underlying skeletal muscles, oral cavity, gastrointestinal tracts, nasal cavities, respiratory systems, ears, eyes, uro-genital system and the brain were all examined for gross pathological lesions and signs of myiasis. Worms within the gastrointestinal tract were collected using thumb forceps and preserved in universal bottles containing 70% ethyl alcohol for characterization and identification.

Tissue sections were collected from areas with gross lesions of CCM. These were skin (from various parts namely the ventral abdomen, flanks, lateral sides of the thighs, scrotal skin), skeletal muscles and draining lymph nodes. Portions of small intestines infested with helminths were sampled and all tissues preserved in 10% buffered formalin.

Adult worms and ectoparasites collected were characterized and identified accordingly. Maggots were also collected and counted for estimation of the load in the dogs. Preserved tissues were transported to histopathology laboratory for tissue processing and analysis using standard methods (Comanescu *et al.*, 2012). After necropsy examination the dog carcasses were disposed following laid down departmental biosecurity measures.

3.9 Laboratory processing and examination of larvae samples

In the laboratory the larvae were washed in distilled water, cleared in 10% sodium hydroxide solution for one hour, washed in distilled water and transferred to 10% acetic acid for 30 minutes and lastly washed in distilled water. They were then dehydrated in ascending concentrations of ethanol (30%, 50%, 70% and 90 %) for thirty minutes in each solution. Thereafter, they were then soaked in absolute alcohol (100%) for one hour and cleared in xylene for one hour. All the internal organs of the maggots were then removed and the posterior spiracles were cut transversely, the anterior end was cut transversely and the thoracic and abdominal body segments cut longitudinally. The specimens were then mounted onto glass slides using DPX mountant and dried in an oven at a temperature of 38°C for 48 hours as described by Almatary and Hassanein (2020). After drying the mounted specimens were then examined under Olympus digital microscope at X40, X100 and X400 magnifications.

3.10 Procedure for culturing of *Cordylobia anthropophaga* larvae and identification of the adult *tumbu* flies

An aluminum foil lining was applied at the base of a plastic dish and dry sand applied to the plastic dish. A piece (0.25kg) of fresh goat liver was then put into the plastic dish container

for the dipteran maggots to feed on during their development and the incubation period before they pupate. Ten live dipteran maggots were freshly collected from the skin of dogs with CCM. The *C. anthropophaga* larvae were then put into the plastic dish container. The plastic dish container was covered using a gauze roll and placed in the open environment in Umuu village, Changwithya East Ward, Kitui Central Sub-county at ambient temperature and exposure to direct sunlight. The development of the maggots into various stages was monitored for 4 weeks (MAFF, 1986).

3.11 Data analysis

Raw data was recorded in Microsoft Excel 2016 spreadsheet and descriptive statistics analysis carried out using Microsoft Excel 2016. Categorical variables of the dogs namely breed, sex, age, type of housing and environmental hygiene were expressed as a percentage (%).

Prevalence of CCM in Kitui County was calculated using the following formula (Margolis *et al.,* 1982).

$$\frac{\text{Total number of dogs infested with CCM}}{\text{Total number of dogs examined}} \times 100$$

Chi-square distribution test was used for analysis of any relationship linking the categorical variables of the sampled dogs and the prevalence of CCM in Kitui County. The categorical variables were considered significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of human respondents in various households

Of the eighty questionnaire respondents, 61.25% were males whereas 38.75% were females. The oldest respondent was 84 years old whereas the youngest was 20 years old with an average age of 42 years. Over sixteen (16.25%) percent of the respondents had primary level of education, 52.50% secondary, 31.25% tertiary whereas 1.25% had University level of education (Table 4.1).

The main occupation of respondents was farming (48.75%) whereas the rest comprised of salaried skilled workers (27.50%), business people (22.50%) and 1.25% were unemployed. Hundred percent (100%) of the respondents were dog owners and were aware about CCM signs in Kitui county. Knowledge on the etiological agent(s) associated with CCM in Kitui County was very low as 16.25% of the respondents were aware about the dipteran flies associated with CCM whereas 83.75% had no idea (Table 4.1).

Table 4.1: Demographic characteristics of human respondents in households in Kitui County

Categorical variable	Qualitative Variable	Number	Percentage (%)
Sex	Male	49	61.25
	Female	31	38.75
Level of education	Primary	13	16.25
	Secondary	42	52.50
	Tertiary	25	31.25
Occupation	Farmer	39	48.75
	Business	18	22.50
	Salaried skilled workers	22	27.50
	Student	1	1.25
Dog ownership	Yes	80	100
	No	0	0.00
CCM awareness	Yes	80	100
	No	0	0.00
Awareness on the	Flies	13	16.25
causative agent of CCM	No idea	67	83.75
Age			
Max = 84 years			
Min= 20 years			
Average age= 41.99 years			

Key

CCM - Canine cutaneous myiasis; **Max -** Maximum; **Min -** Minimum

4.2 Demographic characteristics of dogs in Kitui County

A total of 400 dogs were examined and sampled for CCM infestation. Of these, 59.75% (239/400) were males whereas 40.25% (161/400) were females (Table 4.2). Forty-seven (11.75%) were puppies (below six months), 52 (13%) (between 6-12 months) and 301 (75.25%) were more than 12 months of age (Table 4.2). These dogs comprised of various breeds which included Local dogs (93.75%; 375/400), German shepherd (4%; 16/400), Boerboel (1.25%; 5/400), Japanese Spitz (0.5%; 2/400), Flat coated retriever and Rottweiler each 0.25% (1/400; Table 4.2). The dogs were kept for various reasons namely; security (43.75%), hunting and security (41.25%), pet and security (8.75%), pet (3.75%); and hunting and breeding business (1.25%; Table 4.2).

Table 4.2: Demographic characteristic of dogs sampled in Kitui County

Categorical variable	Qualitative variable	Number of dog	Percentage (%)
Sex	Male	239	59.75
	Female	161	40.25
Age	<6 Months	47	11.75
	6 – 12 Months	52	13.00
	>12 Months	301	75.25
Breed	Local	375	93.75
	German shepherd	16	4.00
	Boerboel	5	1.25
	Japanese spitz	2	0.50
	Flat coated golden retriever	1	0.25
	Rottweiler	1	0.25
Reason for keeping	Security	175	43.75
the dogs	Hunting and security	165	41.25
	Pet and security	35	8.75
	Pet	15	3.75
	Hunting	5	1.25
	Breeding business	5	1.25

4.3 Dog housing structures and hygiene measures

Of all dogs examined only 25% were housed (Table 4.3). They were housed in kennels (25%) and lairs living in dug out holes and earthen floors (75%) (Table 4.3). The frequency of cleaning the housing structures varied from one household to another with 18.75% cleaning

the kennels daily while 7.5% and 73.75% of the dog owners cleaned weekly and rarely, respectively (Table 4.3). Only 18.75% of the housing structures (kennels and lairs) were clean while 81.25% were dirty due to contamination with dog feces and urine (Table 4.4).

Ectoparasites control measures were practiced by 38.75% of the respondents while other dog owners (61.25%) did not control them. They used chemical formulations such as dog shampoos and pour on formulations namely New pal® (Sumithrin), Dudukrin® (Pyrethrin), Bravecto® (Fluralaner), Pestigon® (Fipronil) and sevin® (Carbaryl) in combination with various organophosphate and Pyrethroid formulations to control ectoparasites within kennels namely Ectomin® (Cypermethrin), Delete® (Cypermethrin) , Steladone® (Chlorfenvinphos), Sevin® (Carbaryl) and Norocleanse® (Glutaraldehyde 15%w/v and Coco-benzyl-dimethyl-ammonium chloride 10% w/v (Table 4.3).

Table 4.3: Demographic characteristics of risk factors associated with canine cutaneous myiasis in Kitui County

Categorical variable	Qualitative variable	Number of units	Percentage (%)
Housing	Yes	100	25.00
	No	300	75.00
Type of housing	Kennel	100	25.00
	Lair	300	75.00
	House shared with other animals	0	0.00
Frequency of cleaning the Kennels/environment	Daily	105	26.25
	Rarely	295	73.75
Cleanliness of the dog house / environment	Clean	75	18.75
	Dirty	325	81.25
Common ectoparasite Control measures	Pour on formulations (Pestigon® and sevin®)	26	32.50
	Dog shampoo (Dudukrin®, Bravecto® and Newpal®)	5	6.25
	None	49	61.25
Chemicals for spraying the kennels	Ectomin®	6	7.50
	Delete®	1	1.25
	Steladone®	1	1.25
	Sevin®	10	12.50
	Norocleanse®	8	10.00

Table 4.4: Relationship between housing structures, their hygiene and occurrence of canine cutaneous myiasis in Kitui County

Categorical variable	Q. variable	No. examined	Positive (%)	χ^2	*p. value*
Are the dogs housed?	No	300	178 (59.33)	99.6094	0.0001
	Yes	100	2 (2)		
Frequency of cleaning the dog house	Daily (clean)	75	2(2.67)	66.8386	0.00001
	Rarely (dirty)	325	178(54.77)		

Key

Q. Variable - Qualitative variable; No. examined - Number examined; χ^2 - Chi-square statistic; p-value - Probability value

The prevalence of CCM in the housed dogs and lairs was 2.0% and 59.33%, respectively and this was statistically significant ($p<0.05$) as shown in Table 4.4.

The prevalence of CCM in dogs housed in clean kennels (cleaned-daily) was only 2.67% and statistically significant ($p<0.05$) relative to that of dogs housed in rarely cleaned lairs environment (54.77%; Table 4.4).

4.4 Other animals kept by dog owners

Dog owners in Kitui County kept other animals such as cattle, goats, sheep, chicken, rabbits and donkeys. Cutaneous myiasis infestation in other animals in households was reported by 38.75% whereas 61.25% of the respondents had not observed any infestation. Out of the overall cases of cutaneous myiasis (CM) reported in other livestock, 93.55% were in goat kids and 6.45% in lambs (Table 4.5). Two cases of CM furuncular lesions in goats, one case affecting the flanks in a goat kid causing erythematous furuncular lesions and the other case

affecting scrotal skin of a billy goat causing localized swelling between the scrotal skin and the underlying testicular tissue (orchitis) of the affected testicle (Figure 4.1b). A total of six maggots were recovered from the goat kid flank lesions and the billy goat testicular furuncular lesions.

Table 4.5: Demographic characteristics of other animals in sampled households in Kitui County

Categorical variable	Qualitative variable	Number of households	Percentage (%)
Other animals in the household	Cattle	54	67.50
	Goats	39	48.75
	Sheep	9	11.25
	Chicken	66	82.50
	Rabbits	4	5.00
	Donkeys	14	17.50
Does CM affect other animals?	Yes	31	38.75
	No	49	61.25
Animals affected	Goat kids	29	93.55
	Lambs	2	6.45

Key

CM: Cutaneous myiasis

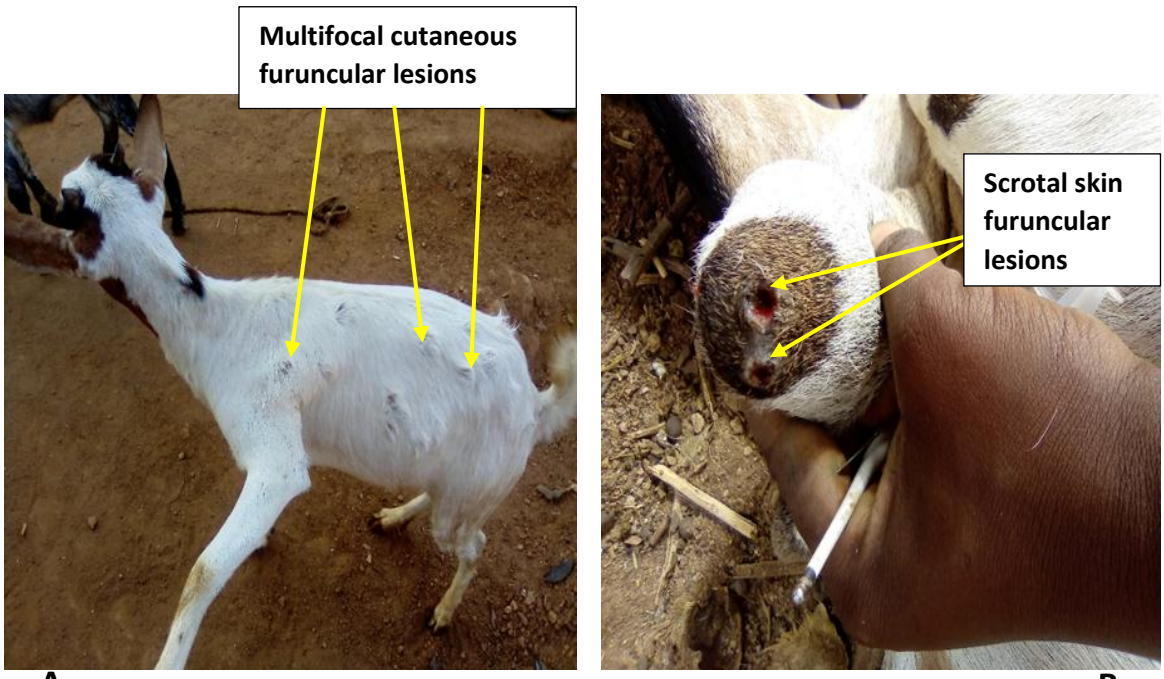

Figure 4.1: A small East African goat kid with multiple furuncular lesions of cutaneous myiasis (A - arrows) and testis with furuncular lesions (B- arrows)

4.5 Zoonotic cutaneous myiasis in Kitui County

Canine cutaneous myiasis was reported to be zoonotic by 93.75% (75/80) of the respondents in Kitui County (Table 4.6).

Table 4.6: Report of human cutaneous myiasis and management in Kitui County

Categorical variable	Qualitative variable	Number of respondents	Percentage (%)
Does CM affect humans?	Yes	75	93.75
	No	5	6.25
Management of HCM	Seeks medical assistance	0	0.00
	Manual removal at household level	75	100

Key

CM: Cutaneous myiasis

HCM: Human cutaneous myiasis

4.6 Diseases of dogs in Kitui County

Various diseases of dogs and their seasonal prevalence in Kitui were reported by dog owners (Table 4.7 and 4.8, respectively).

Table 4.7: Other diseases of dogs, their frequency and seasonal occurrence reported by dog owners in Kitui County

Disease	Frequency	Season
Cutaneous myiasis	Often observed	January to December
Rabies	Rarely	Dry season (April to November)
Scabies	Rarely	January to December
Helminthosis	Often observed	January to December
Canine parvovirus	Rarely	January to December
Canine distemper	Rarely	January to December
Transmissible venereal tumor	Rarely	Breeding season (January to December)
Canine ehrlichiosis (Tick fever)	Rarely	January to December
Canine aflatoxicosis	Often observed	January to December
Infectious canine hepatitis	Rarely	January to December
Pulicosis	Often observed	January to December
Ascites	Rarely	January to December
Dermatophytosis	Rarely	January to December

4.7 Skin diseases of dogs in Kitui County

Various skin diseases of dogs were reported by respondents in Kitui County. Canine cutaneous myiasis in Kitui county referred to by various local names such as `Mienya`, `nzungu` or `sinyu` was the most common (62.50%) skin condition of dogs reported by 100%(80/80) of the dog owners (Table 4.8). The other skin diseases reported included scabies, pulicosis, skin tumors, skin sores, transmissible venereal tumor and dermatophytosis. The dog skin disease frequency, overall percentage and the local names are shown in Table 4.8.

Table 4.8: Common dog skin diseases, number of respondents', frequency of occurrence and their common names in Kitui County

Disease	Respondents'	Frequency	Percentage (%)	Local name
Cutaneous myiasis	80	Often observed	62.5	Mienya/nzungu/sinyu
Scabies	25	Rarely	19.53	Ungùu/musyuliko
Pulicosis	12	Often observed	9.38	Ngala
Skin tumors	4	Rarely	3.17	Kanza
Skin sores	3	Rarely	2.34	Itau
TVT	3	Rarely	2.34	Kisonono
Dermatophytosis	1	Rarely	0.78	Ung'uu

Key: TVT - Transmissible venereal tumor

4.8 Canine cutaneous myiasis infestation management as reported by respondents

Canine cutaneous myiasis infestation was reported by 77.5% of the respondents to be the most common in puppies whereas 33.5% reported it in all dog age groups (Table 4.9). The furuncular lesions of CCM were commonly reported on skin on the ventral areas of the body such as the ventral abdomen, lateral sides of the thighs, vulvar, tail, sternum, shoulders, neck, ears and mouth regions. These body areas are more likely to get into direct contact with the soil when the dogs lay down in the kennel, lair or other areas (Table 4.9).

Manual removal of *C. anthropophaga* larvae by application of gentle digital pressure on the cutaneous furunculoid lesions was the most common action taken by 82.5% of the respondents after noticing that their dogs had CCM. Other respondents sought for Veterinary assistance from the county veterinary offices (8.75%), washed the dogs with soap water (7.50%). Some respondents (1.25%) did not take any action against CCM (Table 4.9).

Over seventy percent (77.5%) of the respondents reported that they only sought veterinary assistance from the county veterinary offices and local agro-vets when CCM infestation was severe whereas, 22.5% of the respondents did not seek veterinary assistance. The disease was mainly managed by treatment by 97.5% whereas 2.5% took no action (Table 4.9). Subcutaneous injection of ivermectin was the drug of choice used by 87.5% of the respondents for the treatment of CCM in Kitui County. Another, 42.5% of the respondents reported Paracetamol® (Acetaminophen), administered orally as the alternative drug of choice for treatment of myiasis although its mechanism of action and efficacy in the treatment of CCM has not been documented (Table 4.9). Other drugs/chemicals used to treat infested dogs were dog shampoo and soap water (Table 4.9). Average duration taken by the dogs for complete recovery from CCM after either subcutaneous injection of ivermectin® or oral administration of paracetamol® (acetaminophen) was 6 days.

Table 4.9: Responses on canine cutaneous myiasis management from respondents in Kitui County

Aspect	Response(s) from respondents	Percentage (%)
Age groups commonly affected		
Puppies	62	77.50
Young adults	0	0.00
Adults	0	0.00
All ages	18	22.50
Actions taken when you notice dog is infested with canine cutaneous myiasis		
Manual removal of the maggots	66	82.50
Seeking veterinary assistance	7	8.75
Washing dogs with soap water	6	7.50
No action	1	1.25
Do you seek county veterinary or local agro-vets assistance?		
Yes	62	77.50
No	18	22.50
How do you manage canine cutaneous myiasis?		
Treatment	78	97.50
No action	2	2.50
Throw away the dog	0	0
Specific drug for treatment of canine cutaneous myiasis		
Ivermectin	70	87.50
None	10	12.50
Any other drug?		
Panadol	34	42.50
Soap water	2	2.50
Dog shampoo	2	2.50

4.9 Prevalence of canine cutaneous myiasis in Kitui County

4.9.1 Sub-county prevalence of canine cutaneous myiasis in Kitui County

The overall prevalence of CCM in Kitui County was 45% (Table 4.10). The highest prevalence of CCM was reported in Kitui Central 65%, Mwingi North 52.5%, Kitui South 48.50%, Kitui Rural 40%, Mwingi Central and Mwingi West each 40%, Kitui West 38.3% and Kitui East 36.70% sub counties, respectively (Table 4.10).

Table 4.10: Prevalence of canine cutaneous myiasis in eight sub-counties of Kitui County

Sub-county	Number of dogs examined	Number of positive cases	% Prevalence
Kitui Central	60	39	65
Mwingi North	40	21	52.5
Kitui South	40	19	48.5
Kitui Rural	60	24	40
Mwingi Central	40	16	40
Mwingi West	40	16	40
Kitui West	60	23	38.3
Kitui East	60	22	36.7
Total	**400**	**180**	**45 (Overall prevalence)**

4.9.2 Gender, breed and age prevalence of canine cutaneous myiasis

The prevalence of CCM in males and females was 42.7% and 48.5%, respectively in Kitui County (Table 4.11). The prevalence of CCM in puppies (<6 months), young adults (6-12 months) and Adults (>12 months) was 51.1%, 34.6% and 45.9% respectively (Table 4.11). However, differences in sex and age groups were not statistically significant ($p>0.05$). The prevalence of CCM in local (mongrels) and pure breed dogs was 47.5% and 8%, respectively (Table 4.11). This difference was statistically significant ($p<0.05$).

Table 4.11: Canine cutaneous myiasis in different sex, age and breed groups of dogs

Variable	**Categorical variable**	**Number of dogs examined**	**Positive cases (%)**	***p.* value**
Sex	Male	239	102 (42.7)	0.2616
	Female	161	78 (48.5)	
Age	< 6 months	47	24(51.1%)	0.2182
	6-12 months	52	18(34.6%)	
	>12 months	301	138(45.9%)	
Breed				
	Local/Mongrels	375	178(47.5%)	0.0001
	Pure breeds	25	2 (8%)	

4.10 Characterization and identification of etiological agent(s) associated with canine cutaneous myiasis in Kitui County

4.10.1 Macroscopic examination of dipterous larvae

Recovered diptera larvae were white to dark brown ranging between 8-12 mm in length, barrel shaped, pointed anteriorly and tapering to a larger posterior end (Figure 4.2). The larvae

had a pair of black oral hooks. The thoracic and abdominal body segments had black minute cuticular spines (Figure 4.3). The posterior end had two posterior spiracles not widely separated.

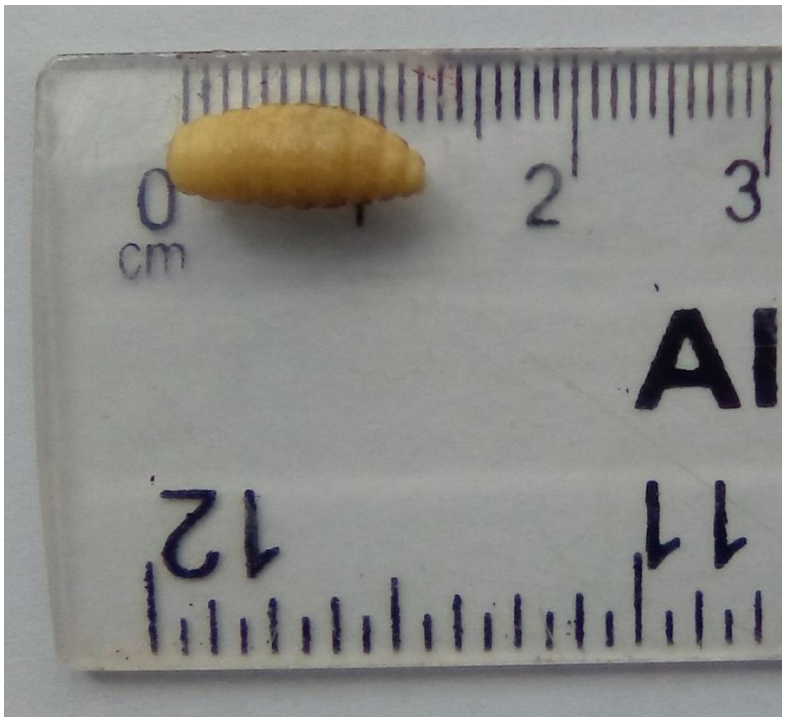

Figure 4.2: Length of *Cordylobia anthropophaga* larvae in centimeters (1.2 cm)

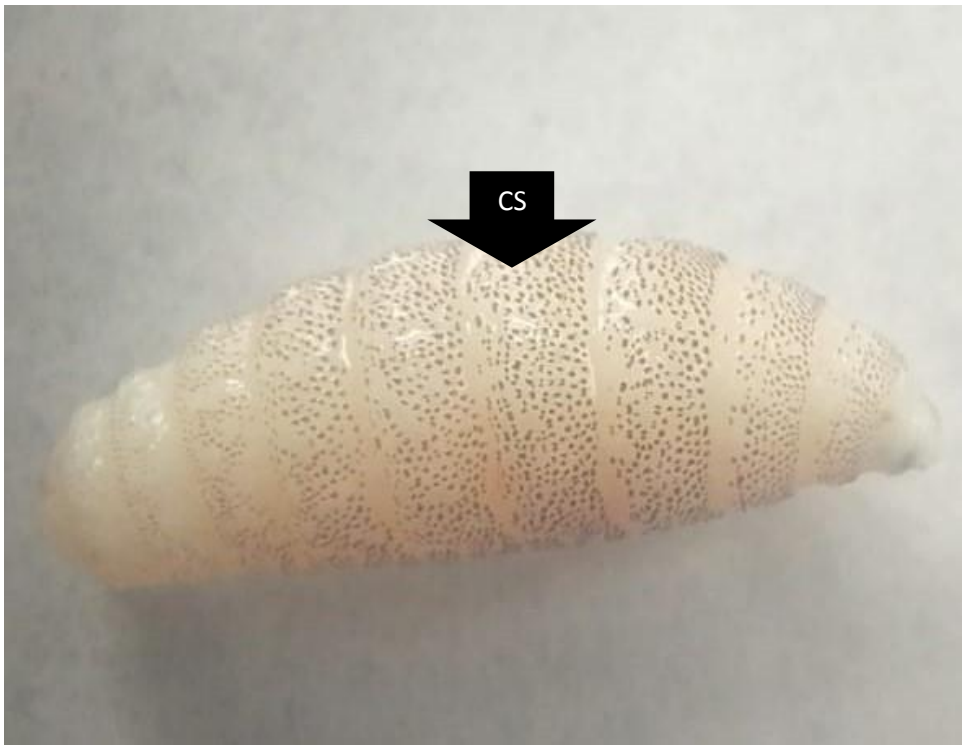

Figure 4.3: *Cordylobia anthropophaga* larvae with a barrel shaped body with dorsal minute black cuticular spines on thoracic and abdominal body segments

4.10.2 Microscopic examination of mounted dipterous larvae

The anterior end of the *C. anthropophaga* larvae had cephalopharyngeal skeleton with pair of oral hooks and short conical brown cuticular spines on the thoracic body segments of the larvae (Figure 4.4a and b).

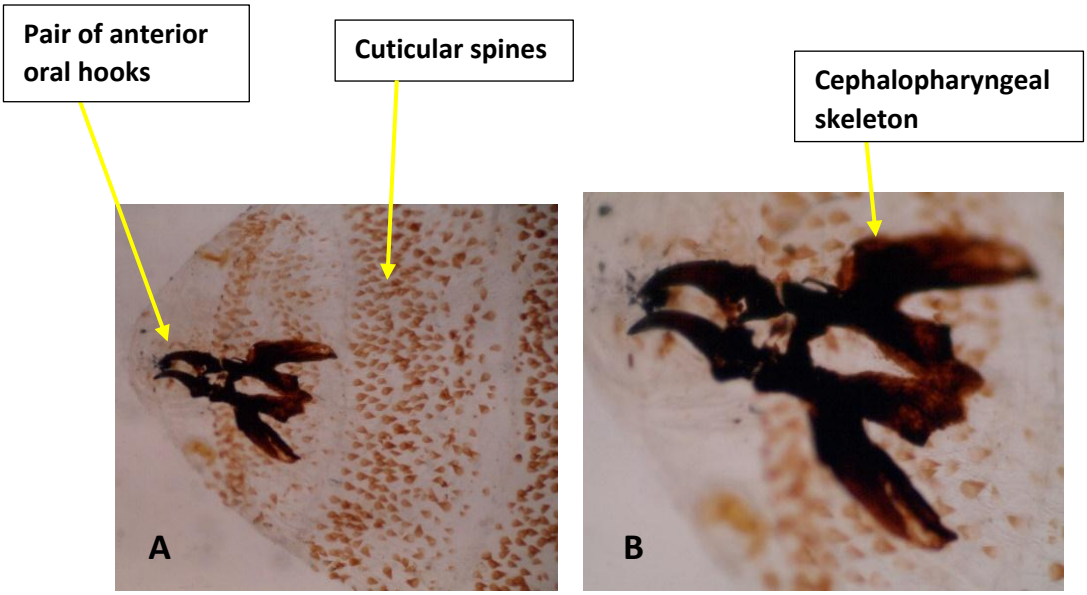

Figure 4.4: The anterior end of *Cordylobia anthropophaga* larvae (A - X40 and B - X100 magnification)

The thoracic and abdominal body segments of the *C. anthropophaga* larvae had posteriorly pointed brown conical cuticular spines (Figure 4.5a and b)

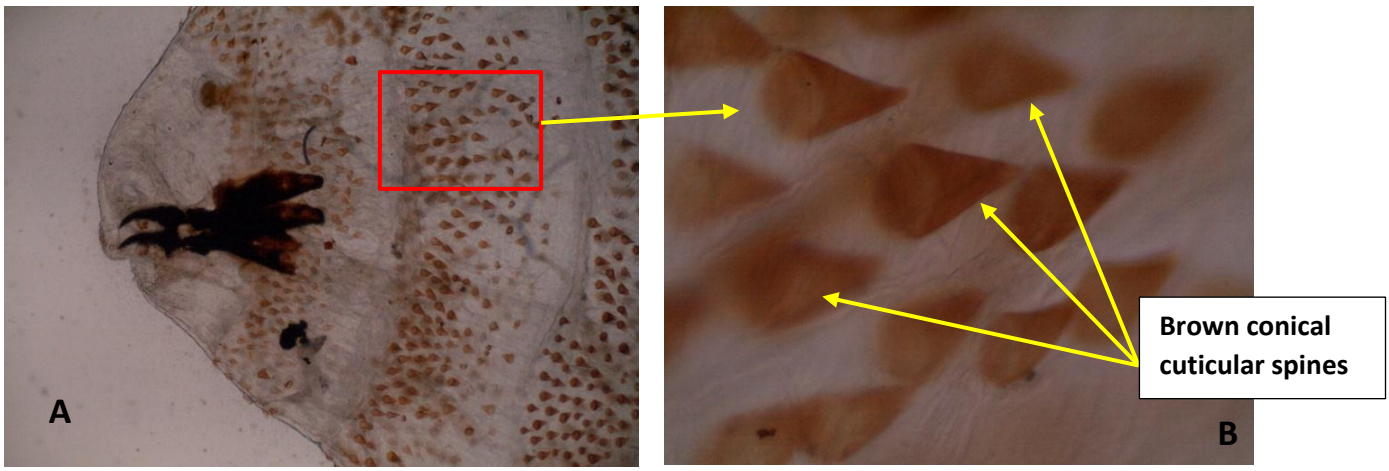

Figure 4.5: Thoracic and abdominal body segments' cuticular spines of *Cordylobia anthropophaga* larvae (A: X40; B: X100 magnification)

The posterior end of *C. anthropophaga* larvae had two posterior spiracles each with three distinct spiracular slits (Figure 4.6a and b).

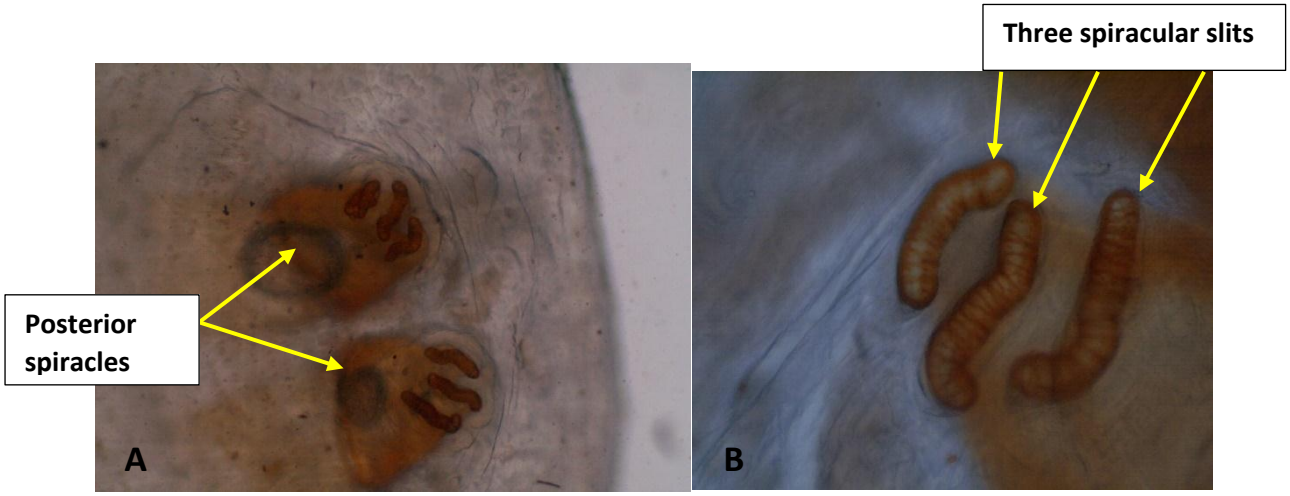

Figure 4.6: Posterior end of *Cordylobia anthropophaga* larvae with two posterior spiracles each with three distinct spiracular slits (A: X40 and; B: X100 magnification)

4.11 *Cordylobia anthropophaga* larval cultures

The pupal stages were dark in color (Figure 4.7 a) whereas the emerging adult `tumbu` flies were yellow brown in color with diffuse grey patches on the thorax and dark color on the posterior part of the abdomen (Figure 4.7 b).

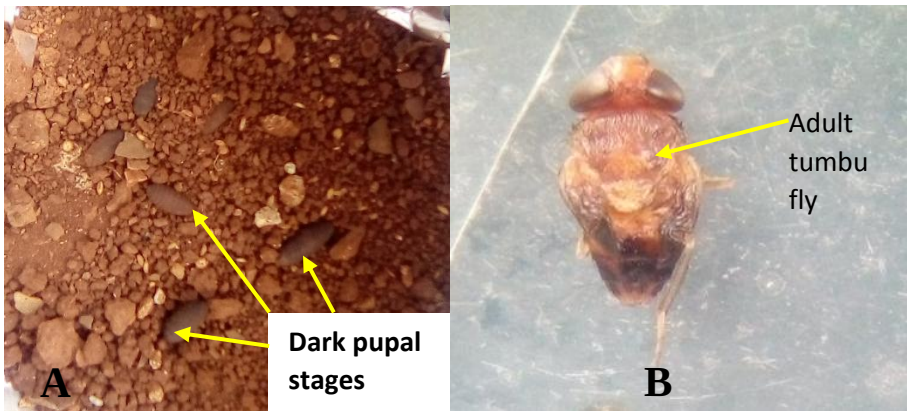

Figure 4.7: Cultured *Cordylobia anthropophaga* pupal (A) and hatched adult *tumbu* fly (B) stages

4.12 Pathological lesions associated with canine cutaneous myiasis in Kitui County

4.12.1 Gross lesions

All the four dogs (100%) had rough hair coats. Two of the dogs (50%), 1.5 months old puppies had concurrent severe fleas (*Ctenocephalides canis*), ticks (*Haemophysalis leachi* and *Riphicephalus sanguineus*) on the skin and gastrointestinal worms (*Dipylidium caninum* and *Toxocara canis*) infestations. They had poor body condition, pale mucus membranes and skeletal muscles indicative of anemia (Figure 4.8).

Figure 4.8: One and half months old puppy showing poor body condition and pale musculature due to anemia

There were pathognomonic lesions for canine cutaneous myiasis in all (100%) dogs characterized by multifocal nodular skin lesions with a 3 mm diameter central pore (furuncular lesions). These were distributed on the ventral abdomen, lateral and medial sides of the thighs, perineum and tail, lateral sides of the chest (flanks), sternum, neck, skin of the ears, vulva mucosa, scrotal skin and the interdigital spaces of the fore and hind limbs.

The furuncular lesions were nodular, erythematous at the base (approximately 1 cm diameter) and had a central pore (approximately 3mm diameter). Some discharged freely, on death of the dog or on application of digital pressure at the base of the furuncles they oozed serous fluid mixed with blood. The hairs around the furuncles were raised and few (alopecia). Some were matted due to sero-hemorrhagic exudate from the opened discharging pore at the center of the lesion.

Yellow-brown maggots emerged from furuncles on application of digital pressure on the skin lesions (figure 4.9). There were scarred, depressed tissue patches of alopecia in areas where the lesions had already healed after the maggots have dropped from the host especially on ventral abdomen, flanks and lateral sides of the thighs.

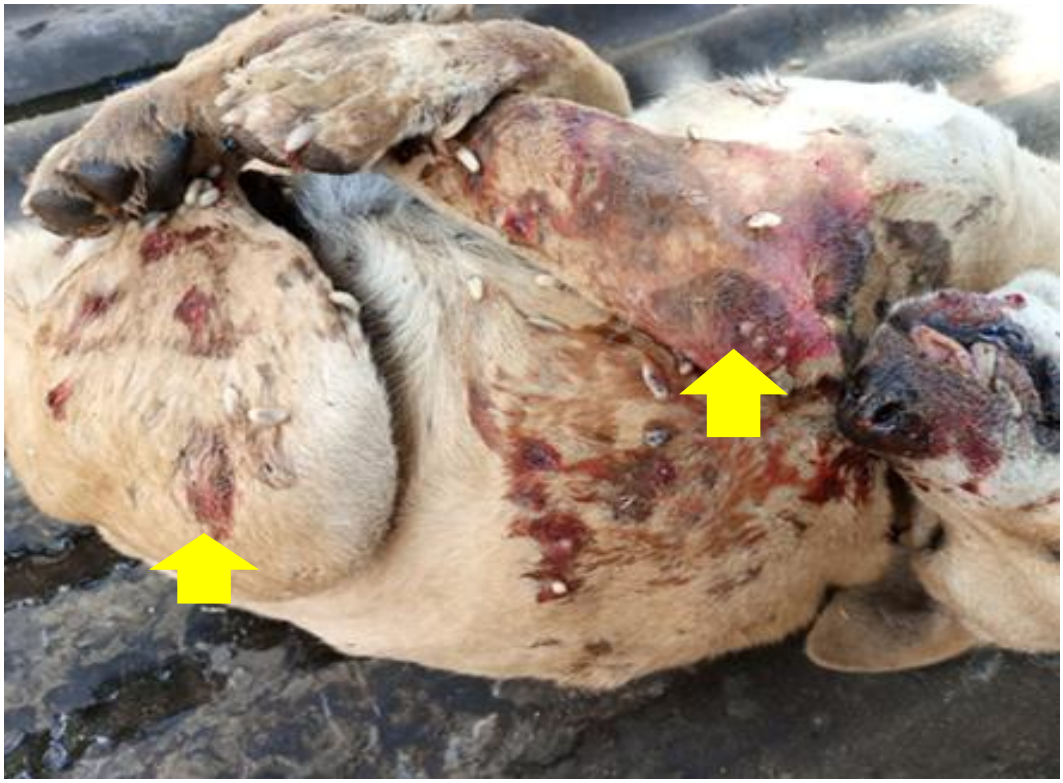

Figure 4.9: Multifocal furuncular skin lesions and hairs matted with sero-sanguineous discharges mixed with blood and grayish to yellow brown *Cordylobia anthropophaga* maggots leaving the 8 years old male dog few hours after euthanasia (arrows)

On skinning the carcasses, the furuncular lesions extended throughout the skin thickness from epidermis to the subcutis. They were characterized by firm consistency, cutaneous edema and congestion (Figure 4.10). The underlying skeletal muscles were bright red due to hyperemia. The draining lymph nodes especially the pre-scapular and popliteal lymph nodes in the affected regions were enlarged (regional lymphadenopathy) in all the four dogs (100%) euthanized for necropsy examination.

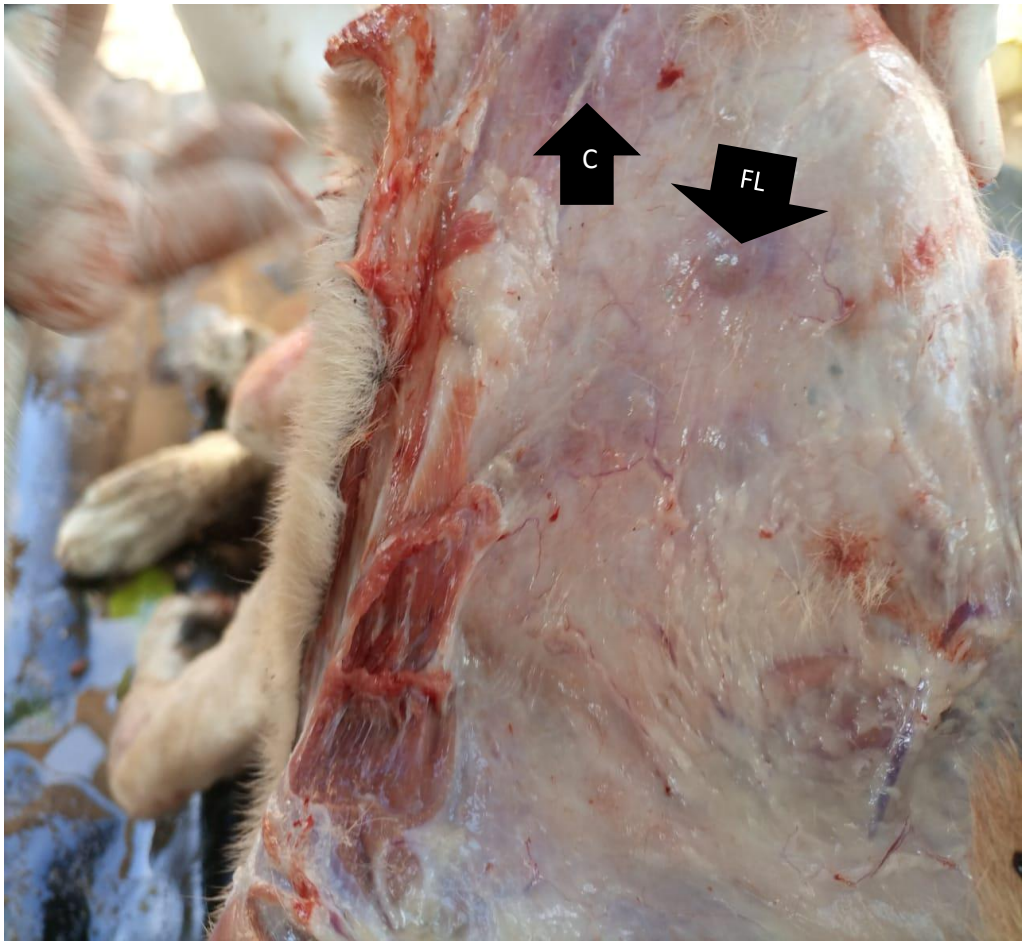

Figure 4.10: Multifocal furuncular lesions (FL) extending throughout the thickness of the skin and generalized congestion (C)

There were *C. anthropophaga* maggots mixed with stomach and duodenal contents without notable reaction on the cardiac sphincter, stomach and duodenal mucosae (pseudo myiasis) in one of the dogs (Figure 4.11). There were no significant lesions in the other body organs, systems and tissues.

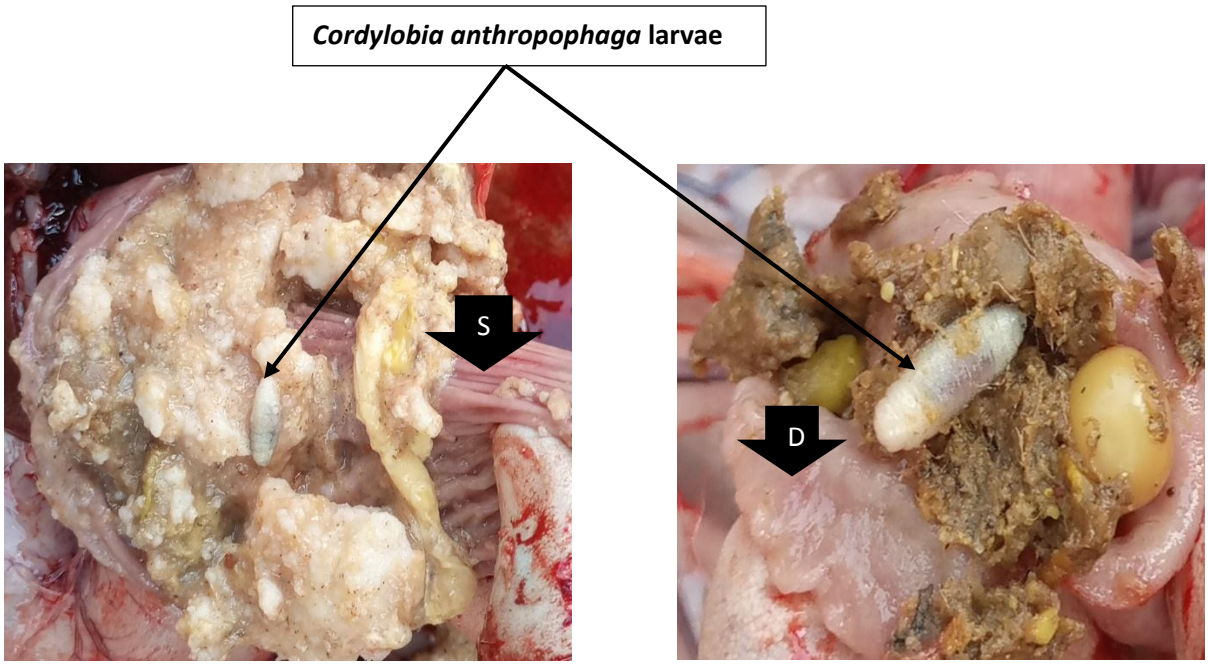

Figure 4.11: *Cordylobia anthropophaga* larva in the stomach (S) and duodenal (D) contents in an 8 years old dog

4.12.2 Microscopic lesions

Microscopically, there was disruption, discontinuity and desquamation of the stratified squamous epithelium of stratum corneum of the affected skin. Thickening of the epidermal layer (acanthosis) of the affected skin (Figure 4.12a). Multifocal circular cavities with *C. anthropophaga* larvae were observed in the dermis and extended into the subcutaneous tissue of the skin (Figure 4.12b). There was also dilatation of adjacent blood vessels with red blood cells (Figure 4.12c). The dermal circular (parasitic granuloma) lesions were surrounded by a fibrous capsule with a mixed inflammatory cellular infiltrate with mononuclear cells and numerous eosinophils as the predominant inflammatory cells (Figure 4.12d).

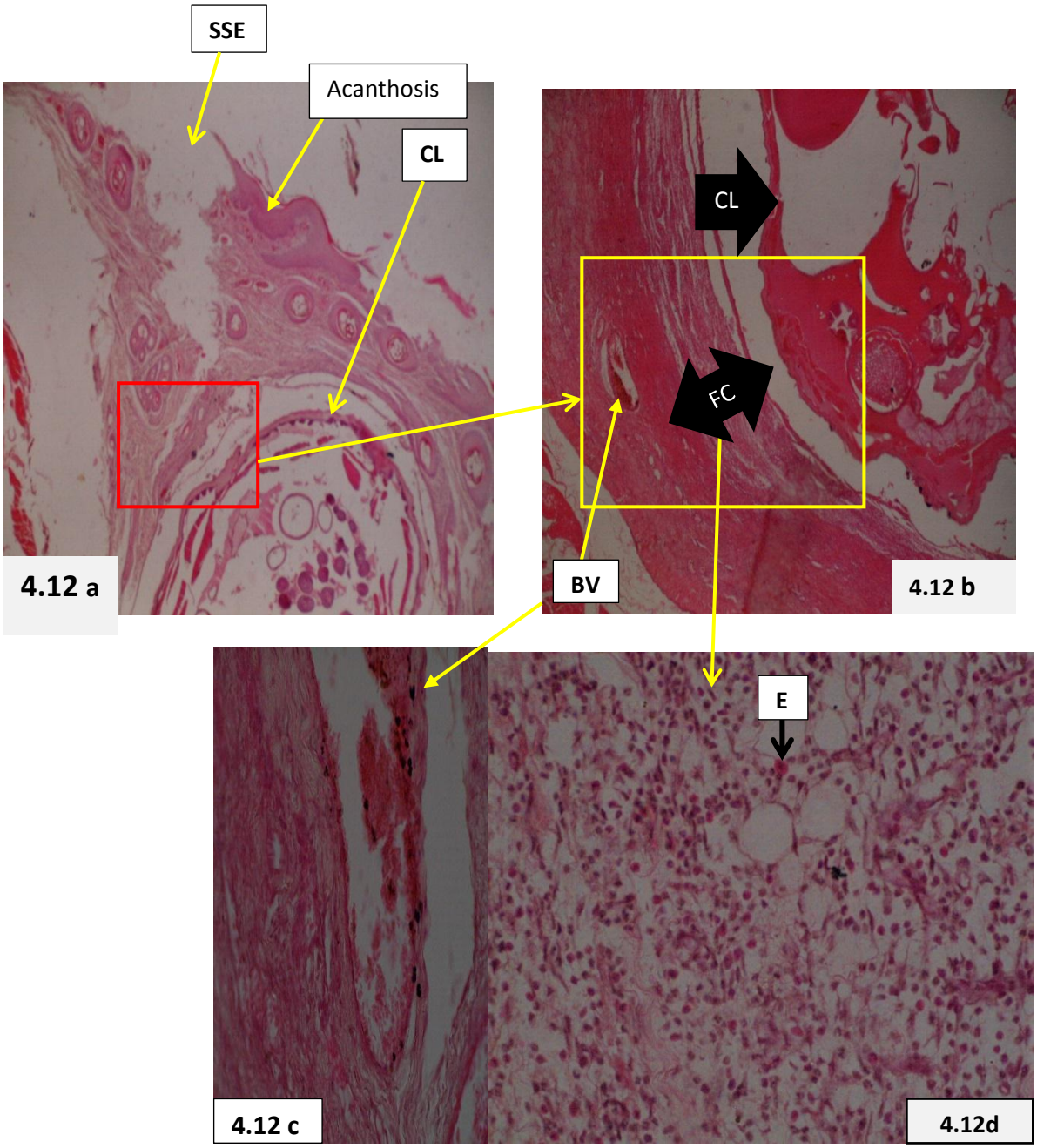

Figure 4.12: Histological sections of *Cordylobia anthropophaga* infested dog skin showing**: a)** disruption, discontinuity and desquamation of stratified squamous epithelium (SSE), acanthosis and *Cordylobia anthropophaga* larvae (CL) section lodged within the dermis (H&E; X40); **b)** circular dermal lesions with *Cordylobia anthropophaga* larvae (L) encapsulated by fibrous capsule (FC); **c)** dilated dermal blood vessels (**BV; H&E; X100)**; **d)** Mixed cellular infiltrate within the fibrous capsule with predominant eosinophil (E) inflammatory cells (**H&E; X400)**

There was destruction of the normal architecture of the underlying subcutaneous skeletal muscles showing edema and coagulative necrosis (Figure 4.13a). In some areas there was proliferation of fibroblasts and subsequent deposition of eosinophilic collagen fibres with mixed inflammatory cellular infiltrate in the fibrous tissue. Eosinophils were the predominant inflammatory cells (Figure 4.13b).

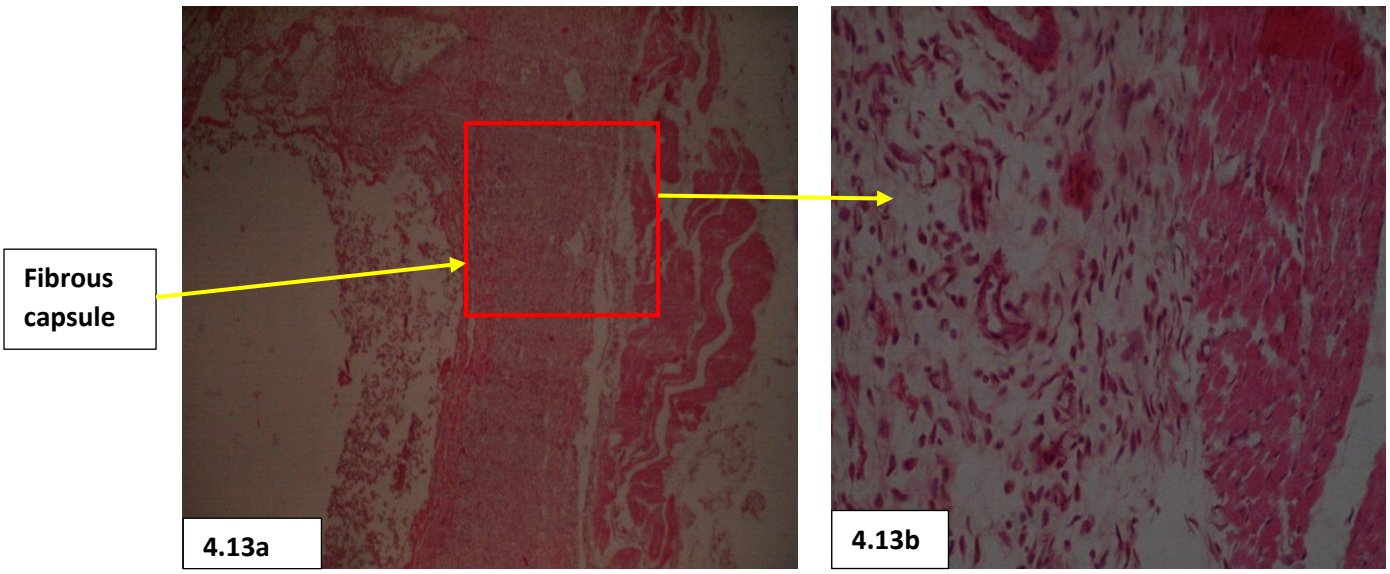

Figure 4.13: Histological sections of *Cordylobia anthropophaga* infected dog skeletal muscles showing: **a)** coagulative necrosis of the affected skeletal muscles and deposition of fibrous capsule (H&E; X100) and; **b)** fibroplasia and mixed inflammatory cellular infiltrate within the fibrous tissue with predominant eosinophil cells (H&E; X400)

Regional lymph nodes draining affected areas had massive infiltration of eosinophils and plasma cells within the sub-capsular sinus, germinal centers and trabecular sinuses extending to the medullary sinuses. The lymph nodes also had reactive germinal centers with moderate infiltration of foreign body giant cells into the germinal centers (Figure 4.14 a-d).

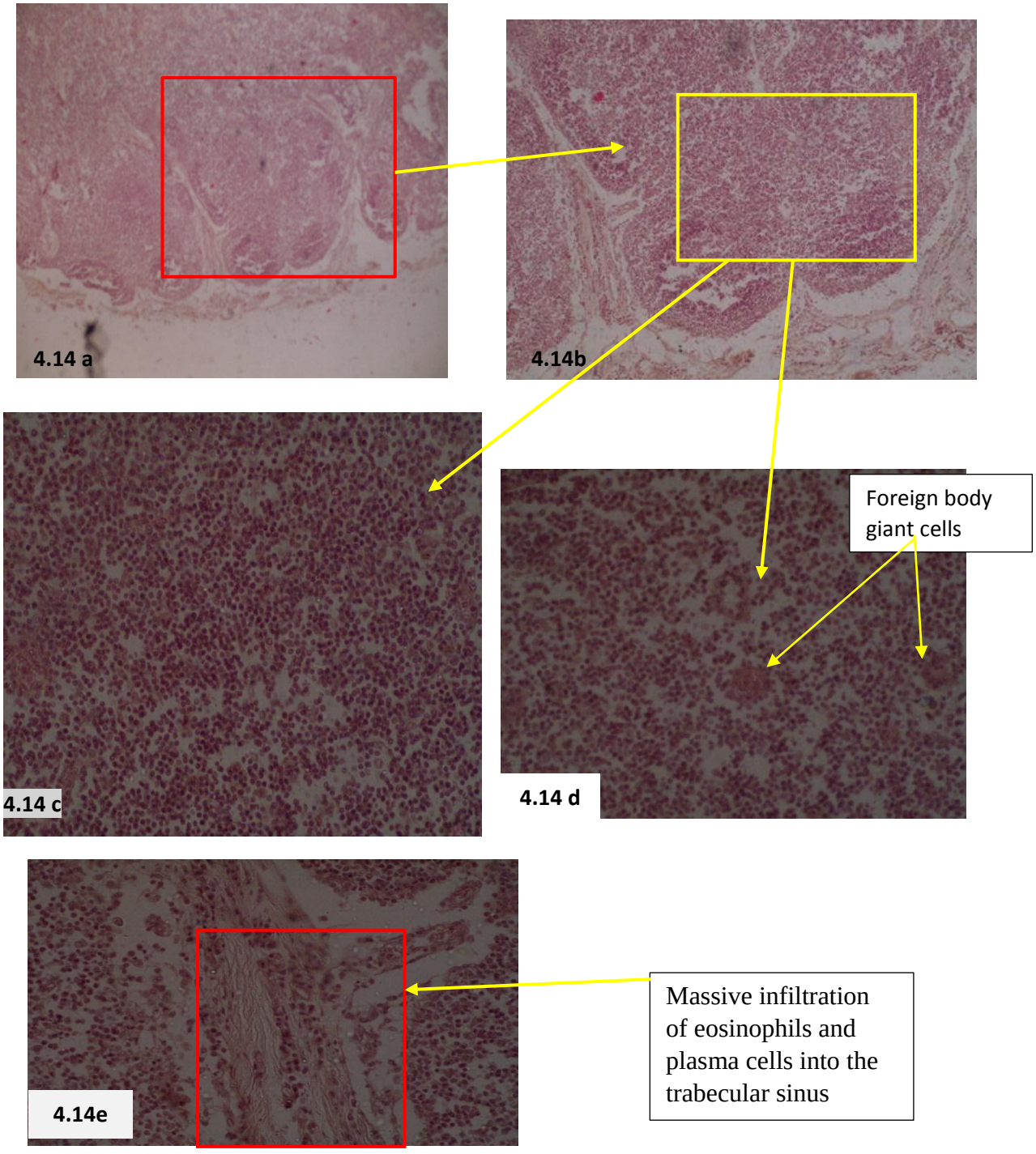

Figure 4.14: Histological sections of enlarged prescapular lymph nodes from dogs infected by canine cutaneous myiasis showing: **a)** subcapsular sinus, germinal centres and trabecula sinus (**H&E; X40**); **b)** mixed cellular infiltration into the germinal centres (**H&E; X100); c)** massive infiltration of predominant eosinophils and plasma cells into the germinal

centers(**H&E; X400**; d) eosinophils and plasma cells and moderate infiltration of giant cells into the germinal center(**H&E; X400**) and; **e)** infiltration of eosinophils and plasma cells into the trabecular sinus(**H&E; X400**)

4.13 Other lesions observed on the carcass

4.13.1 Lesions caused by helminths in sampled dogs

Two puppies had adult worms of *D. caninum* and *T. canis* worm infestation in the intestines. Grossly the intestinal mucosa was hemorrhagic and lumen had hemorrhagic intestinal contents mixed with adult worms (Figure 4.15a and b).

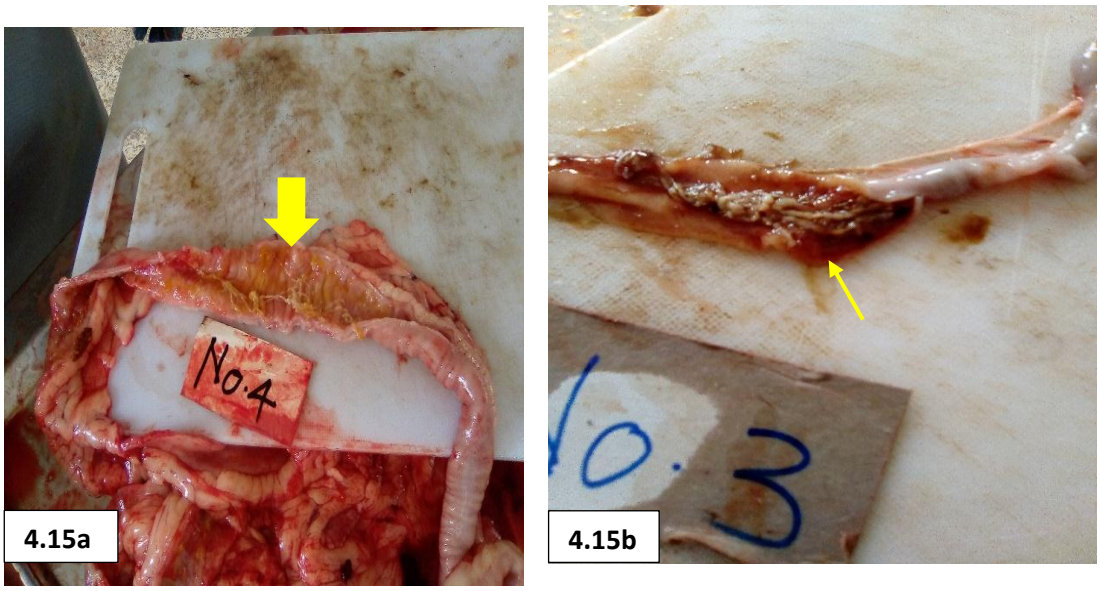

Figure 4.15: Gross lesions of the small intestines of dogs infested with *Dipylidium caninum* and *Toxocara canis* worms showing: **a)** Thick corrugated intestinal mucosa of dog no. 4 with fibrinous exudate and injected blood vessels on the serosa surface; and b) a loop of the small intestine of dog no. 3 with hemorrhagic mucosa, hemorrhagic intestinal contents with adult worms blocking the intestines (yellow arrows)

Microscopically there was atrophy, sloughing off and destruction of the small intestinal villi (Figure 4.16a). There was also mixed cellular infiltration into the intestinal villi, underlying submucosa and the inner circular muscular layer of the small intestines with eosinophils being predominant cells (Figure 4.16 b and c).

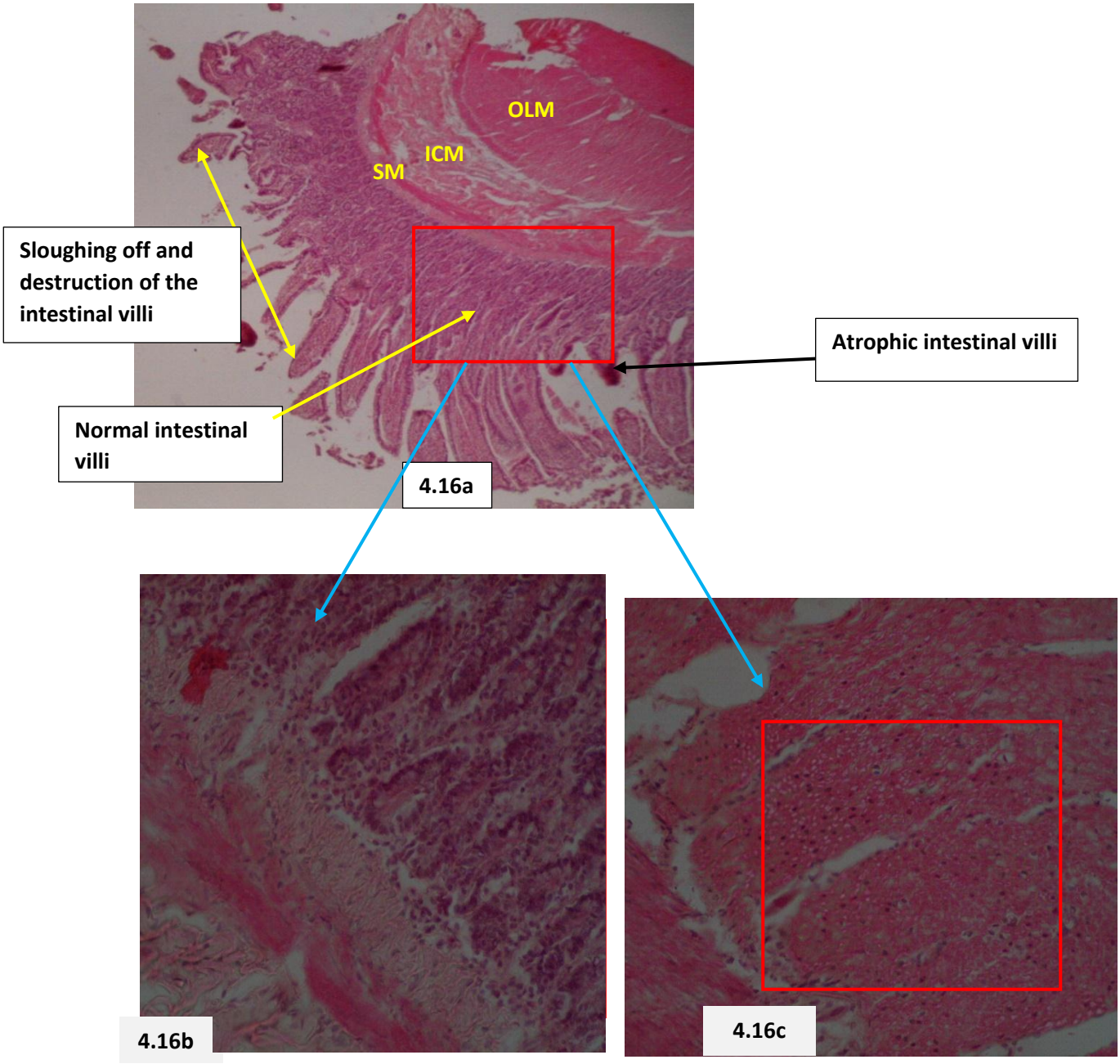

Figure 4.16: Histological sections of small intestines from dogs infected with *Dipylidium caninum* and *Toxocara canis* worms showing: a) atrophy, destruction and erosion of the intestinal villi (**H&E; X40)**; a) mixed cellular inflammatory infiltrate predominantly eosinophils into the intestinal villi and the underlying (**H&E; X400**); c) inflammatory cells mainly eosinophils in the inner circular muscle layer (**H&E; X400**)

Key
M- Mucosa; **SM-** Submucosa; **ICM-** Inner circular muscle layer; **OLM-** Outer longitudinal muscle layer

4.13.2 *Toxocara canis*

The worms' head was elliptical due to presence of the large cervical alae and the mouth was surrounded by three lips (Figure 4.17) whereas on the posterior end, the tail had a terminal narrow appendage (Figure 4.18).

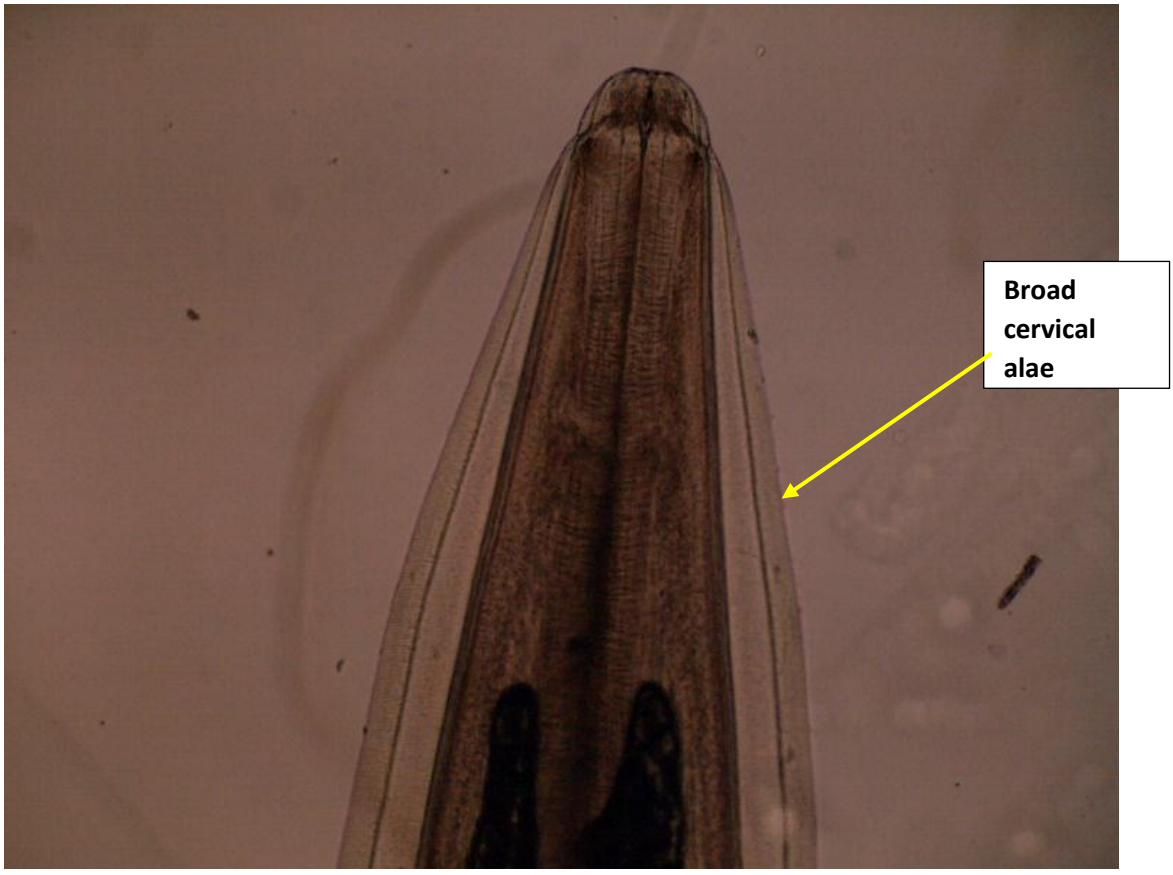

Figure 4.17: Anterior end of *Toxocara canis* with prominent cervical alae

Figure 4.18: Posterior end of *Toxocara canis* with a terminal narrow appendage

4.13.3 *Dipylidium caninum*

The scolex on the anterior end had two suckers and rostellum with rows of small black hooks (Figure 4.19). Gravid proglottids of *D. caninum* were as shown in Figure 4.20.

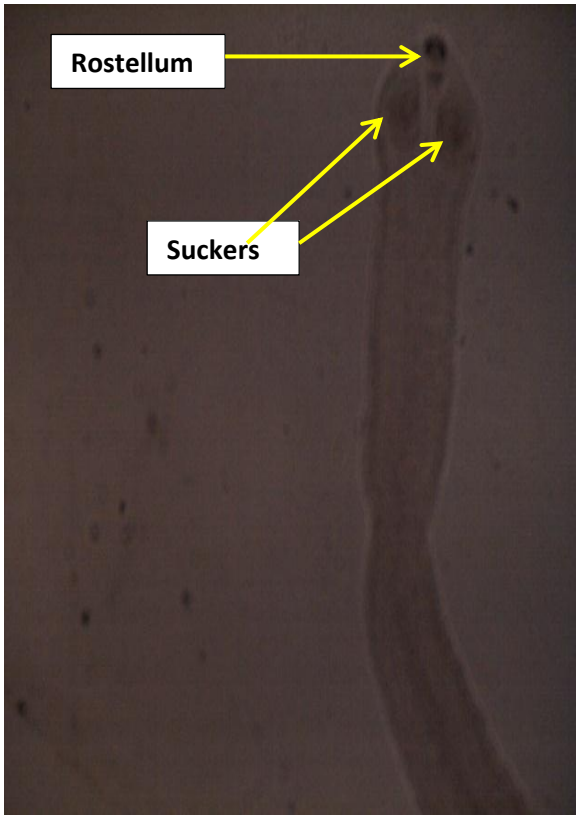

Figure 4.19: Anterior end of *Dipylidium caninum* showing a rostellum and suckers

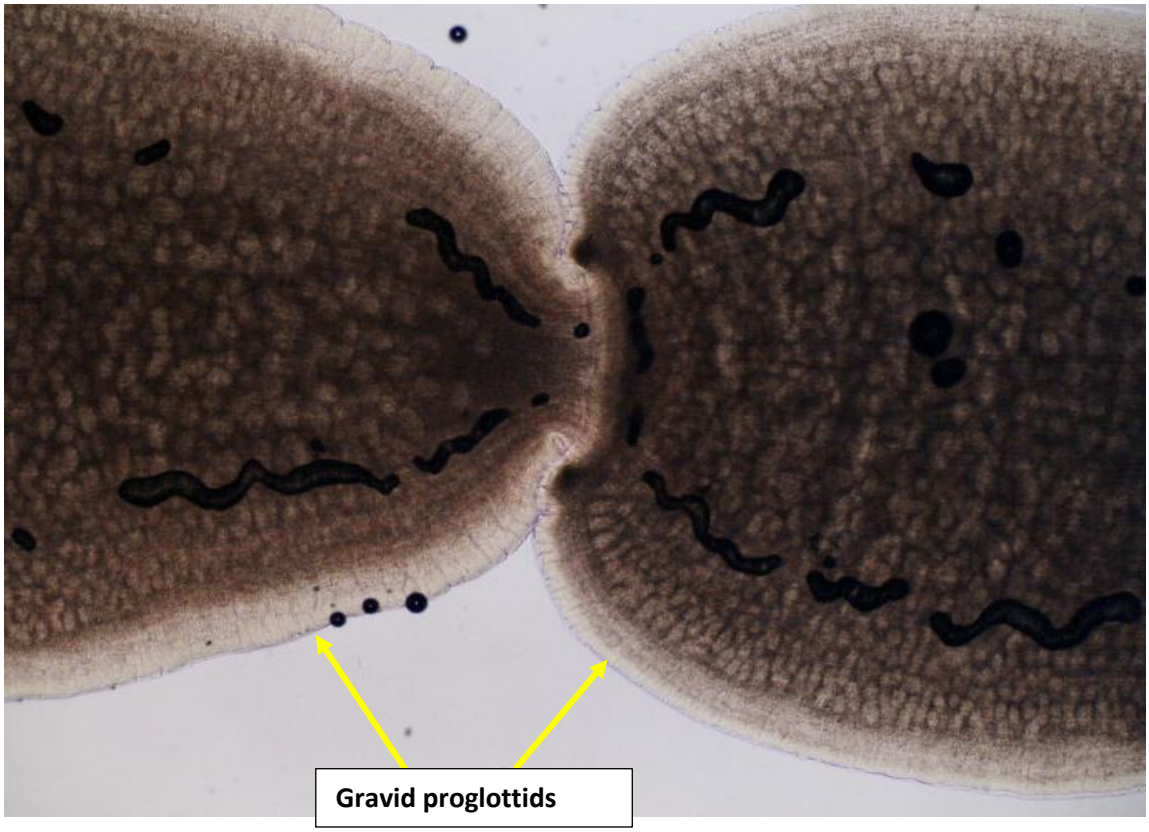

Figure 4.20: Gravid proglottids of *Dipylidium caninum*

4.14 Ectoparasites observed on sampled dogs in Kitui County

Fleas and ticks were observed infesting dogs during necropsy examination.

4.14.1 *Rhipicephalus sanguineus*

The ticks had hexagonal basis capitulum with short hypostome, short broad palpi and festoons (Figure 4.21).

Figure 4.21: *Rhipicephalus sanguineus* tick found on sampled dogs from Kitui County

4.14.2 *Ctenocephalides canis*

The fleas had both genal comb and pronotal comb on the anterior end, the head was rounded anteriorly and the first spine of the genal comb was shorter than the other spines**.** Sensilium was present on the posterior end (Figure 4.22).

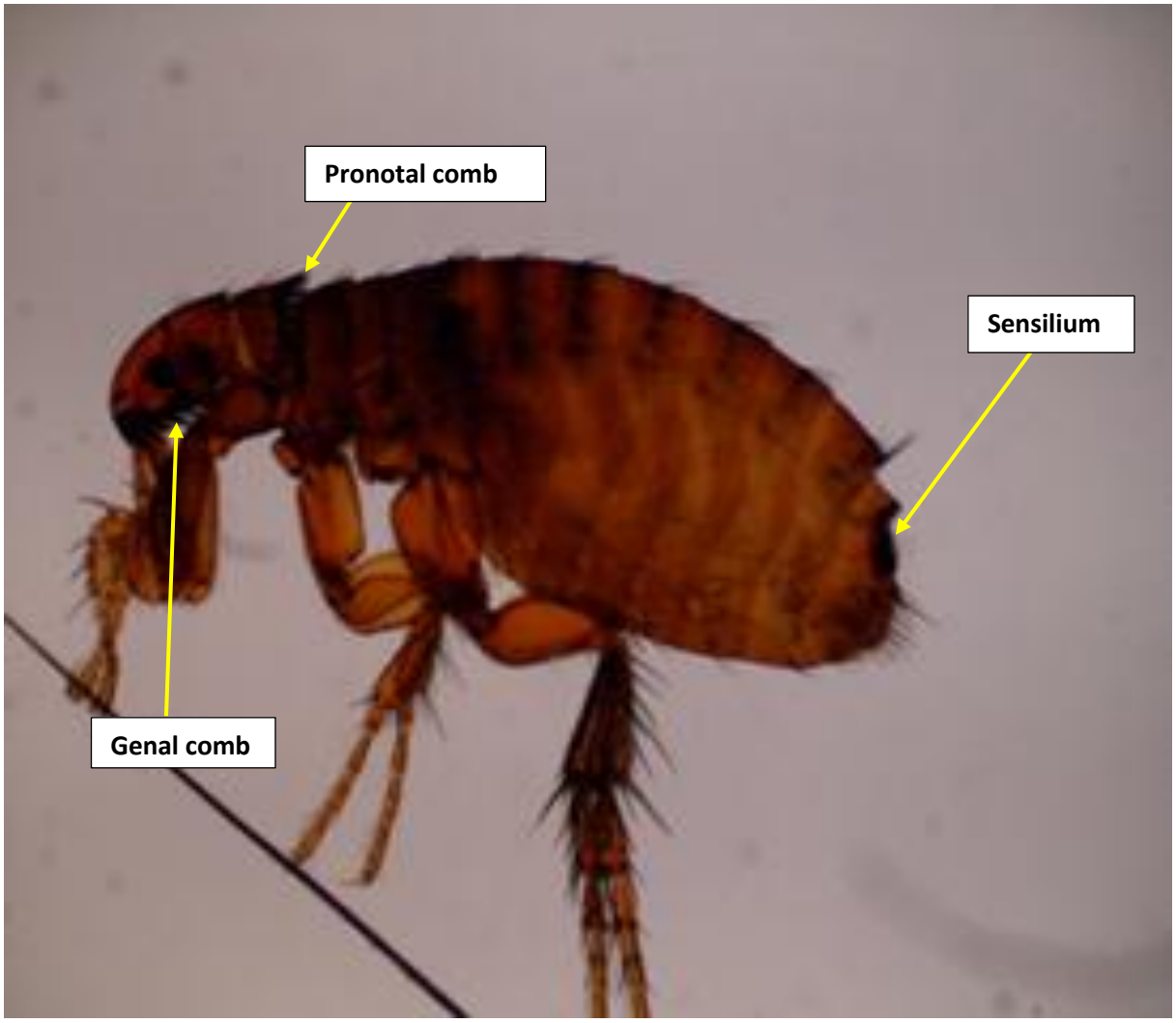

Figure 4.22: *Ctenocephalides canis* flea found on sampled dogs from Kitui County

4.14.3 *Haemaphysalis leachi*

The ticks were not ornate, had a rectangular basis capitulum and their palpi were wider than long (Figure 4.23).

Figure 4.23: *Haemaphysalis leachi* tick found on sampled dogs from Kitui County

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The present study is the first confirmed report of canine cutaneous myiasis (CCM) in Kitui County, Kenya. The overall prevalence of canine cutaneous myiasis in the current study was found to be 45%. There are no published articles or recorded research works in Kenya to compare with but the results are in agreement with Abebe (2017) who reported an overall prevalence of 42.19% in dogs in Ethiopia. It differs conspicuously with previous studies by Johnson *et al.* (2016) in Ghana and Ogo *et al.* (2005) in Nigeria who reported prevalence of canine cutaneous myiasis of 7.4% and 75.80% respectively. The differences in the overall prevalence could be attributed to differences in geographical zones, prevailing environmental climatic conditions and dogs' management practices. Kitui central had the highest sub-county prevalence (65%) of CCM in the county. Kitui central sub-county lies in the highland areas of Kitui County which receive high amounts of rainfall compared to the drier lowlands which receive low amounts of rainfall (Anonymous, 2015). These findings are in agreement with those of Urquhart *et al.* (1996) who reported that high ambient temperature, humidity, rainfall intensity and host susceptibility are the main factors which determine the occurrence of clinical myiasis in both humans and animals.

There was no sex predisposition to canine cutaneous myiasis since both male and female dogs were affected equally by the disease. However, prevalence was higher in the female sex group in Kitui County but was not statistically significant ($p>0.05$). This differed with Abebe (2017) who reported sex predisposition in female dogs to canine cutaneous myiasis in Ethiopia.

Out of the dogs infested with canine cutaneous myiasis, 98.89% were mongrels (local breed) suggesting breed predisposition to the disease since the difference in breed was statistically significant ($p<0.05$). This compares with Johnson *et al.* (2016) who reported an 82.80% prevalence among the mongrels in greater Accra in Ghana. However, they differed with findings of Abebe (2017) who reported no breed predisposition to canine cutaneous myiasis in Ethiopia. Low incidence of canine cutaneous myiasis in pure breeds may be attributed to the fact that pure breeds get special care such as proper housing and routine hair coat grooming to control ectoparasites using pour on formulations and dog shampoos (Johnson *et al.*, 2016; Singh, 2020).

Among the various dog age groups, the young puppies (<6 months) were the most infected with myiasis (51.06%). This was as previously reported by Ogo *et al.* (2005) in Nigeria; Johnson *et al.* (2016) in Ghana and Abebe (2017) in Ethiopia at prevalence of 68.95%, 93.10% and 71.56% respectively, although it had a lower prevalence. Abebe (2017) reported that low prevalence of canine cutaneous myiasis in adult dogs is attributed to low exposure to the ground where the eggs of "tumbu fly" are laid. Unlike young dogs who are routinely in contact with ground and associated higher heat dissipation that may attract the fly larvae hence the disease.

The prevalence of canine cutaneous myiasis (45%) in Kitui county might be an underestimation of the disease in the county because only 11.75% (47/400) of the physically examined dogs were below 6 months of age, yet young dogs have been reported to be more susceptible to the condition (Kozminska, 1981). The infested dogs were in poor body condition with rough hair coats matted with serous discharges from the multifocal

erythematous furuncular skin lesions. There were also patches of alopecia more prominent on lateral thighs and on the flanks where the furuncular skin lesions had already healed as reported by Johnson *et al.* (2016). The furuncles were distributed on the ventral abdomen, lateral and medial sides of the thighs, perineum and tail, vulva lips in females and scrotal skin in males, lateral sides of the chest (flanks), sternal region, neck, skin of the ears and interdigital spaces of the fore and hind limbs. These findings were in strong agreement with previous studies done by Singh (2020) that reported that ventral body parts like vulvar lips and perianal region are more vulnerable to the attack of flies because these regions frequently come in contact with the ground while the dog is sitting and become soiled with excreta and the dipteran maggots irritate host animals resulting in anorexia and poor health. Parasitized animals often become restless and rubs the affected areas. This study concurred with previous work by Leschnik *et al.* (2008) who reported that *Cordylobia anthropophaga* larvae infestation produces lump-like subcutaneous lesions with a central hole in the skin of human and animals.

Lack of housing and proper hygiene were the major risk factors for the exposure and occurrence of canine cutaneous myiasis in dogs in the study area. The disease was higher in unhoused dogs (59.33%) where the environment was rarely cleaned and was dirty during study period. Johnson *et al.* (2016) reported that unhoused dogs (82.8%) were more likely to easily move about in the community in search for food and could be exposed to the dipterous flies leading to the development of myiasis. Yanuartono *et al.* (2019) reported that dogs are predisposed to myiasis infestation by low level of animal hygiene, cage and environment hygiene. Anderson and Huitson (2004) reported that neglect of pets with subsequent accumulation of urine and feces is one of the primary risk factors for the development of canine cutaneous myiasis. The housing and hygiene measures of the dog environment were

significant ($p<0.05$) risk factors that determined the occurrence of canine cutaneous myiasis in housed dogs in this study area. Cleanliness and regular disinfection of sleeping places is significant in the control of canine cutaneous myiasis (Soulsby, 1986).

The other animals kept by the respondents in the study area included; chicken, goats, sheep, cattle, donkeys and rabbits but cases of cutaneous myiasis due to *Cordylobia anthropophaga* larvae infestation were only reported in goat kids and lambs. This is because goat kids and lambs do not accompany does and ewes to the grazing fields, they are left around the homestead where they mingle with puppies and seek shelter in dog kennels. This is in agreement with Juyena *et al.* (2013) who reported high prevalence of cutaneous myiasis in young ruminants aged < 6 months in Bangladesh. Two cases of cutaneous myiasis infestation due to *Cordylobia anthropophaga* larvae were encountered affecting a goat kid and testicles in a breeding billy goat. The affected goat kid had rough hair coat with hairs matted with serous discharges from furuncular skin lesions. The affected billy goat had swollen scrotal skin and testicle (orchitis) which could result to poor fertility and associated economic loss to the farmer due to infertility. Such cases were not observed by other researchers Johnson *et al.* (2016) in Accra, Ghana, Kitui by Mugachia (2018) and Bangladesh by Juyena *et al.* (2013).

Canine cutaneous myiasis was reported as the most common skin disease of dogs in the study area. The condition was previously reported to be endemic (Mugachia, 2018) in the study area. The common management practices for canine cutaneous myiasis included manual removal of dipteran larvae from the furuncles by application of digital pressure by 82.50% but there was no action taken by 17.50% of the respondents towards the management of the infestation. Only 8.75% of the respondents sought veterinary assistance from the local County

veterinary offices and local agrovets whereas 91.25% of the respondents did not seek veterinary assistance. The alternative management practices included use of soap water and dog shampoos. These management practices have not been previously reported in Kenya.

The standard treatment of cutaneous myiasis focuses on antibiotic therapy concomitantly with mechanical removal of necrotic tissues (Arruda *et al.*,2017). Ivermectin which belongs to avermectins group and is a semisynthetic macrolide antibiotic (Arruda *et al.*,2017) is used.

The drug is widely used for the treatment and control of ectoparasites and endoparasites infestations in large animals, such as cattle, horses, sheep, goats, and camelids and the drug is generally administered as a single dose of 0.4-0.6mg/kg body weight (Arruda *et al.*,2017). Since the drug is rapidly absorbed and reaches high blood concentrations within a relatively short period, the larvae are quickly expelled from the wound (Arruda *et al.*,2017). Dogs in the study area were reported by respondents to heal from the cutaneous myiasis infestation 5 days after ivermectin treatment although it has not been documented. Paracetamol® (acetaminophen;N-(4-hydroxyphenyl)acetamide,N-(4-hydroxyphenyl) ethanamide), human formulation prescribed as a painkiller in humans was the alternative drug used commonly by the residents and claimed to be effective in the treatment of canine cutaneous myiasis in the study area although its dosage rate and mechanism of action in the treatment of this condition is unknown and need further investigations. All the respondents (100%) were aware about the occurrence of canine cutaneous myiasis in the study area but knowledge about the etiological agent(s) associated with the canine cutaneous myiasis in the study area were very low with only 16.25% of the respondents having knowledge about the etiological agent(s) whereas 83.75% of the respondents had no idea about the etiological agent(s) associated with CCM in the study area. This study has confirmed the etiological agent to be *C. anthropophaga.* These

findings suggest that lack of knowledge on the etiological agent(s) and control measures for canine cutaneous myiasis play a role in the high prevalence of canine cutaneous myiasis in the study area.

Cases of *Cordylobia anthropophaga* infestation in human cutaneous myiasis (HCM) were also reported and observed in the study area. Almatary *et al.* (2020) reported that human cutaneous myiasis should be expected in people living in poor hygienic conditions and is more frequently seen in rural regions where people are in close contact with pets, as in Kitui. This highlight the public health significance of canine cutaneous myiasis as a zoonotic disease in the study area and further studies are required.

The findings of the present study were in strong agreement with earlier work by Ogo *et al.* (2009) and Abebe (2017) who reported 100% *Cordylobia anthropophaga* larvae infestation associated with canine cutaneous myiasis in Nigeria and Ethiopia respectively. It however, differs with Johnson *et al.* (2016) who reported *Cordylobia rodhaini* and *Dermatobia hominis* as the principal etiological agents of canine cutaneous myiasis.

Cordylobia anthropophaga infestation is associated with significant pathological lesions. The pathognomonic lesions for canine cutaneous myiasis in all necropsy dogs (100%) were multifocal one (1) cm nodular skin lesions with a 3 mm diameter central pore (furuncular lesions). These lesions were distributed on the ventral parts of the body. The findings of this study concurred with previous work by Johnson *et al.* (2016) who reported that anemia, unthriftness, alopecia, lethargy, emaciation and anorexia were the main clinical signs presented by dogs affected by canine cutaneous myiasis in the Greater Accra in Ghana. There are no reports on the gross and microscopic pathological lesions associated with canine cutaneous myiasis, elsewhere as reported in this study.

The adult helminths collected from the small intestines of the puppy were identified as *Toxocara canis* and *Dipylidium caninum* respectively. All the fleas collected from the four dogs were identified as *Ctenocephalides canis*. These findings were in strong agreement with previous work by De-La-Rosa-Arana *et al.* (2018) who reported that *Toxocara canis* is the most prevalent worm in puppies. *Toxocara canis* occurs in the small intestines of the dog and fox (Soulsby, 1986) as observed in the current study. These could have contributed to poor body condition and anemia. It also indicates poor dog management practices.

Dogs infested with both *Dipylidium caninum* and *Toxocara canis* present with general unthriftiness with dull or harsh hair coat, a potbellied appearance or tucked up abdomen, intermittent diarrhea, emaciation, restless, constipation and anemia (Soulsby, 1986). Some of this were observed in the present study; namely, emaciated and anemic puppies infested with both adult *Toxocara canis* and *Dipylidium caninum* helminths. Death usually occurs due to acute intestinal obstruction (Soulsby, 1986), which was observed during necropsy in this study. Grossly the intestinal mucosa was thickened, hemorrhagic and the intestinal lumen had hemorrhagic intestinal contents mixed with adult worms. Microscopically, there was erosion of the villous mucosa and thickening of the intestinal sub-mucosa. There was also mixed cellular inflammatory infiltration into the intestinal villi, sub mucosa and the muscular layers predominantly by eosinophils.

Fleas and ticks have masticatory mouth parts suck blood from their hosts. Infested animals become restless, lose condition, anemic and spoil their coats by biting and scratching (Soulsby, 1986). This can attract dipteran flies to the site resulting in myiasis. In addition to their direct effects on the host animals, the recovered dog ectoparasites were of great veterinary and medical significance. *Ctenocephalides canis* is the intermediate host for *Dipylidium caninum* (dog tapeworm) which is zoonotic and the filarial worm of dog

Dipetalonema reconditum (Soulsby, 1986). *Rhipicephalus sanguineous* (brown dog tick) is the vector for the transmission of tick fever (*Ehrlichia canis)* in dogs (Leschnik *et al.*, 2008). *Haemophysalis leachi* (yellow dog tick) lives on the head and body of its host and transmits canine piroplasmosis (Soulsby, 1986). Dogs appear to play a major role in the spread of cutaneous myiasis to other animals and human in the study area. The ecto- and endo-parasitism indicates poor dog management practices in dogs with cutaneous myiasis.

In conclusion, the results of the present study revealed that CCM is widespread in the study area with an overall prevalence of 45%. It is also evident that dogs, goat kids and lambs in the study area are affected by cutaneous myiasis. The findings of the present study also indicate that dogs are housed in different housing structures with different hygienic measures with less emphasis to the predisposing factors. This may be attributed to lack of knowledge on the etiological agent (s) and control measures for canine cutaneous infestation. *C. anthropophaga,* the `tumbu` fly or `mango` fly larvae are the principal etiological agents for CCM in Kitui County and cause significant pathology in affected dogs.

5.2 Conclusions

1. Dogs in the study area had CCM with an overall prevalence of 45% and was the main canine cutaneous condition in the county.
2. Lack of housing: dog and poor environmental hygiene are the main predisposing risk factors for occurrence of CCM in Kitui County.
3. *Cordylobia anthropophaga* maggots of the tumbu fly were confirmed to be the etiological agent causing CCM in Kitui County.

4. *Cordylobia anthropophaga* maggot infestation caused significant pathology (gross and microscopic) in the affected dogs.
5. Goats and sheep were also diagnosed to be infected with cutaneous myiasis in Kitui County.

5.3 Recommendations

1. Increased awareness of risk factors of CCM in Kitui County is recommended to ensure wellbeing of the host.
2. Further studies on seasonal prevalence, different ecological zones, etiological agent(s) in other counties, public health importance and control strategies of CCM in Kenya are recommended.

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APPENDICES

Appendix 1: Ethical clearance by University of Nairobi, Faculty of Veterinary Medicine, Biosafety, Animal use and Ethics committee

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Kenya.

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Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2021/303

Dr. Nichodemus Kamuti
Dept Vet Pathology, Microbiology, Parasitology
University of Nairobi
02/07/2021

Dear Dr. Kamuti,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Prevalence, Risk factors and Pathological lesions associated with canine cutaneous myiasis in Kitui County, Kenya.

Nichodemus Kamuti J56/34754/2019

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 17th June 2021. We have reviewed your application for ethical clearance for the study. The number of dogs to be sampled, sampling protocol and laboratory tests to be undertaken meets the minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 2: Questionnaire administered to dog owners

Dear respondent,

I am a postgraduate student of the Faculty of Veterinary Medicine, Nairobi University.

I am carrying out a research project on outbreaks of canine cutaneous myiasis ("nzungu") in Kitui County. This is for fulfillment of the requirements for the award of Master`s degree in Veterinary Pathology and diagnostics in the Faculty of Veterinary Medicine, University of Nairobi.

This research questionnaire is for collecting data to provide relevant information required with utmost sincerity. All the information provided will be treated with confidentiality and privacy is guaranteed.

Ward/village……

Sub-county……

GPS coordinates: ……

Date: ……

Questions

Biodata of the respondent

1. Age of the respondent.....

2. Gender; Male (….) Female (…)

3. Highest level of education

Level of education	Tick where applicable
I. Primary level	
II. Secondary level	
III. Tertiary level (College or University)	

4. Profession.....

Biodata of the Dog(s)

5.Do you have a dog(s)? Yes (….) No (…….).

6.If yes how many?.....

7. Age of your dog(s)

Category	Age in years	Male(s)	Female(s).
(I). Adult dogs (>12 months)			
(ii) Young adults (6-12 months)			
(iii)Puppies (<3			

Months)			

8. What are the reasons for keeping the dog(s)?

(i) Pet (……)

(ii) Pet and Security (…….)

(iii) Security (…….)

(iv) Hunting and Security (…….)

(v) Hunting (…….)

(vi) Breeding business (……..)

8(a). Are the dogs housed? Yes (…..) or No(…….)

9. If yes, What is the type of housing

(i) Kennel (…..)

(ii) Lair (…..)

(iii) House shared with other animals (….)

10. If yes, how frequent do you clean the dog kennels? (Tick where applicable)

(i) Daily (…….).

(ii) Weekly (……).

(iii) Monthly (…….).

(iv) Rarely (……).

(v) Never (……)

11. How is the cleanliness of the dog house or lair? Clean (……) Dirty (……)

12. Which ectoparasite control measures are practiced by the respondent? (tick where applicable).

Dog shampoo? …… Pour on formulations?..... Spraying the dog? Kennel hygiene?.....

Any other (specify)……

13. Other animals in the household

Cattle (….)

Goats (….)

Sheep (….)

Chicken (….)

Rabbits (….)

Pigs (….)

Donkeys(….)

Diseases of Dogs

14. Which are the common diseases which affect dogs in this area?

	Disease	Frequency	Season it occurs
I			
II			
III			
IV			

15.Which are the common skin diseases observed in dogs in this area?

	Disease	Frequency	Common name
I			
II			

III			
IV			

16. Are you aware of canine cutaneous myiasis ("Nzungu") infestation in dogs?

Yes..... No.....(Tick where applicable).

17. What causes canine cutaneous myiasis ("Nzungu") in dogs?.....

.....

18. How many of your dogs are infested with canine cutaneous myiasis ("nzungu")?

Number: .....

19. Which age groups are commonly affected by canine cutaneous myiasis ("nzungu")? (Tick where applicable).

Category	Tick where applicable
Adult dogs (>1 year)	
Young adults(3-12 months)	
Puppies (<3 Months)	

20. Which parts of the dog`s body are commonly affected by canine cutaneous myiasis ("Nzungu") nodular lesions?.....

……

21. Which months and season of the year is/are associated with canine cutaneous myiasis ("Nzungu") outbreaks? (Tick where applicable).

(i) November to January (long rain season)?.....

(ii) February to March (short dry season)?.....

(iii) April to May (short rain season)?.....

(iv) June to October (long dry season)?.....

(v) Whole year (January to December)?.....

22. Which action(s) do you take when you notice that the dog(s) have canine cutaneous myiasis("Nzungu")?.....

23. Do you seek for assistance from the local veterinary offices or agro-vets?

Yes (…….) No (.….)

24. How do you manage canine cutaneous myiasis ("nzungu") infestation in dogs?

Treatment (…..)

If treatment, which specific drug do you use for the treatment? ……

Any other method (specify): …….

25. How long does it take for the dogs to recover from canine cutaneous myiasis ("nzungu") after treatment?.....

26. Does canine cutaneous myiasis ("nzungu") affect other animal species in the household?

If yes, which ones……

27. Does canine cutaneous myiasis ("nzungu") affect humans? Yes (…….). No (…….).

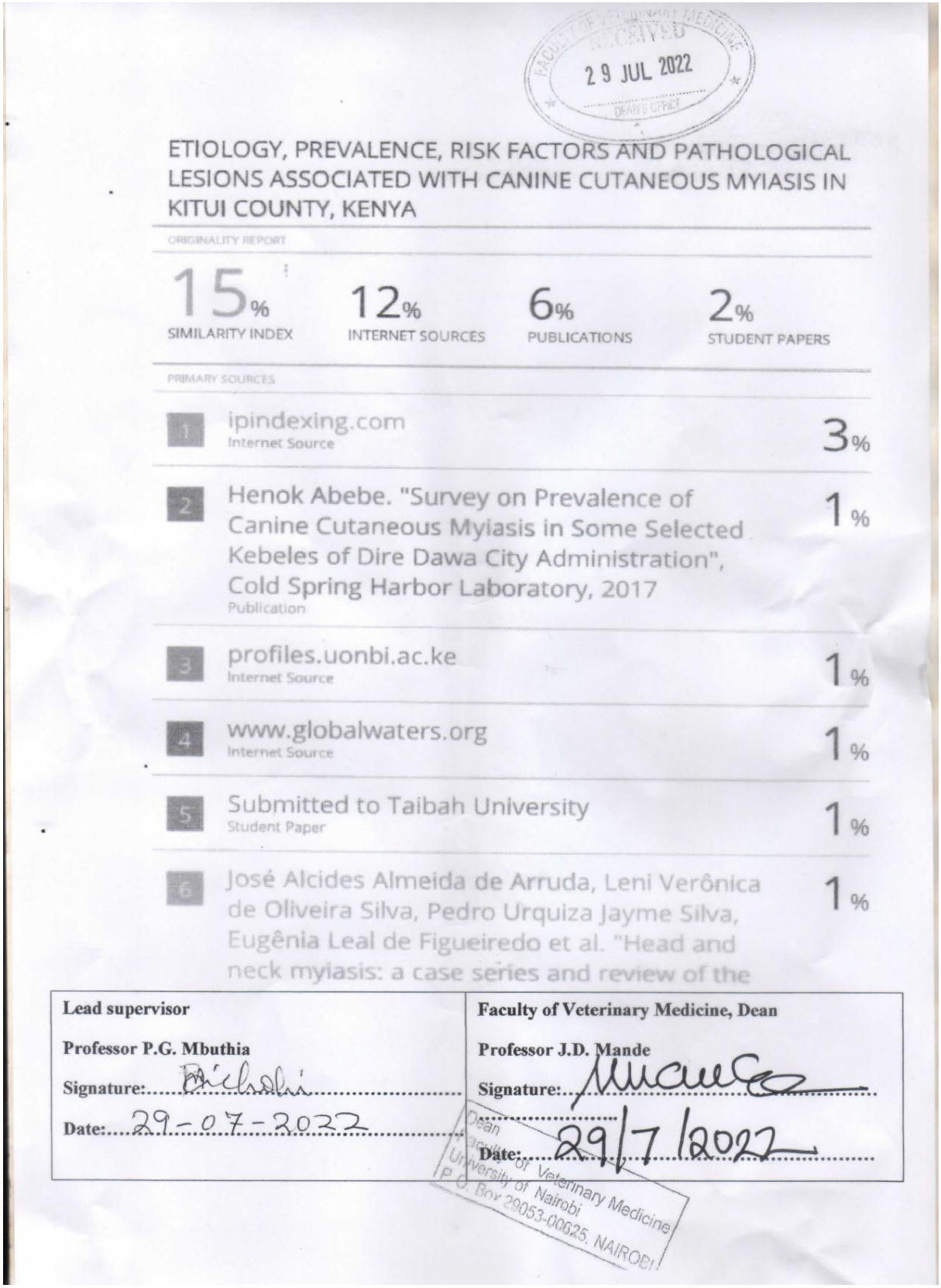
28.If yes how do you manage canine cutaneous myiasis ("nzungu") in humans?.....

29. Which control measures do you observe to prevent/control the myiasis infestation in your dogs and other livestock?.....

Thank you

Dr. Nichodemus Mutinda Kamuti (BVM, UoN).

Appendix 3: Plagiarism report



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ETIOLOGY, PREVALENCE, RISK FACTORS AND PATHOLOGICAL LESIONS ASSOCIATED WITH CANINE CUTANEOUS MYIASIS IN KITUI COUNTY, KENYA

ORIGINALITY REPORT

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6	José Alcides Almeida de Arruda, Leni Verônica de Oliveira Silva, Pedro Urquiza Jayme Silva, Eugênia Leal de Figueiredo et al. "Head and neck myiasis: a case series and review of the	1%

Lead supervisor	Faculty of Veterinary Medicine, Dean
Professor P.G. Mbuthia	Professor J.D. Mande
Signature:	Signature:
Date: 29-07-2022	Date: 29/7/2022

Dean
Faculty of Veterinary Medicine
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