PREVALENCE AND RISK FACTORS ASSOCIATED WITH LINGUATULA SERRATA INFECTION IN DOGS IN ZARIA AND ENVIRONS, KADUNA STATE, NIGERIA

BY

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DECEMBER, 2015

DECLARATION

I declare that the work in this dissertation titled **"Prevalence and risk factors associated with *Linguatula serrata* infection in dogs in Zaria and environs, Kaduna State, Nigeria"** was performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

KHADIJAH ALIYU ABUBAKAR

Name of Student | Signature | Date

CERTIFICATION

This dissertation, titled "PREVALENCE AND RISK FACTORS ASSOCIATED WITH *LINGUATULA SERRATA* INFECTION IN DOGS IN ZARIA AND ENVIRONS, KADUNA STATE, NIGERIA" by Khadijah Aliyu ABUBAKAR meets the regulations governing the award of the degree of Masters of Science Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, and has approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Linguatula serrata is a zoonotic parasite causing visceral and nasopharyngeal linguatulosis in human beings. Dogs are important source of infection for man. This study determined the prevalence of *L. serrata* in dogs in Zaria and environs. Three hundred and ninety nine (399) samples each of faecal and nasal swabs and 360 heads were collected from household and slaughtered dogs respectively and evaluated for the presence of *L. serrata*. Zinc sulphate floatation technique at 1.18 specific gravity and direct smear were used for detection of eggs in faecal and nasal secretions respectively, while heads of slaughtered dogs were split longitudinally and visually inspected for the presence of the parasite in nasal cavities. *Linguatula serrata* eggs were found only in 10 (2.5%) of the 399 nasal secretions from household dogs. There was no statistical association between the sex, age, breed and purpose for which dogs were kept and infection with *L. serrata* ($P > 0.05$). There was also no statistically significant association between occurrence of *L. serrata* and feeding of raw meat (OR 0.966, 95% CI 0.141-1.824), scavenging (OR 0.968, 95% CI 0.948-0.988) and restriction of dog (OR 0.966, 95% CI 0.948-0.988). There was a statistically significant association between infection and raising dogs with other animals ($P= 0.035$) and dogs sampled based on ward ($P= 0.001$). Majority of the dog-owning households (55.2%) are families with the respondents having at least secondary school education in 68.7% of the households. Most respondents (45.7%) own only one dog, and 56.1% never utilized veterinary services. Frequent sneezing and unusual material from the nostrils were reported by 31.3% and 3.1% of respondents respectively. Other parasites eggs found in faeces of household dogs include *Taenia* spp (14.7%), *Isospora* spp (6.0%), *Ancylostoma* spp (5.0%) and *Spirocerca* spp (4.3%). Although no adult form of *L. serrata*

was observed from slaughtered dogs and also no egg was detected in faecal sample examined, the results from this study indicates dogs that are infected with *L. serrata* in Zaria and environs albeit at low levels of carriage, therefore there is possibility of human infection with *L. serrata* in the area which is of public health importance.

TABLE OF CONTENTS

Title……..……..……..……... i

Declaration.....ii

Certification iii

Acknowledgement iv

Abstract.....vi

Table of Contents……..…viii

List of Tables……xii

List of Plates……xiii

List of Figures……..…xiv

List of Appendices……xv

CHAPTER ONE: INTRODUCTION.....1

1.1 Background of the Study.....1

1.2 Statement of Research Problem 4

1.3 Justification 6

1.4 Aim and Objectives.....7

1.4.1 Aim 7

1.4.2 Objectives 7

1.5 Research Questions.....7

CHAPTER TWO: LITERATURE REVIEW.....9

2.1 Biology of Pentestomids 9

2.2 Morphology of Pentestomids 10

2.3 Reproduction.....11

2.4 Life Cycle of *Linguatula serrata*.....11

2.4.1 Definitive host of *Linguatula serrata* 12

2.5 Incubation Period of *L. serrata* 14

2.6 History of *Linguatula serrata* 14

2.7 Epidemiology **15**
2.8 Risk Factors for Infection **16**
2.9 Pentastomiasis in Nigeria **17**
2.10 Predilection Site for the Parasite **17**
2.11Clinical Features and Pathogenesis **18**
2.12 Transmission **20**
2.12.1 Visceral linguatuliasis 20
2.12.2 Nasopharyngeal linguatuliasis 21
2.13 Symptoms in Man **22**
2.14 Pentestomiasis and Cancer **23**
2.15 Diagnosis of *Linguatula serrata* **24**
2.16 Treatment……..……..**25**
2.17 Prevention and Control of *Linguatula serrata* Infection **26**
2.18 Economic Importance for Humans **27**
2.19 Distribution of Pentastomiasis **28**
2.20 Cases Reported **29**
2.20.1 Australia 29
2.20.2 Germany 29
2.20.3 Ecuador 30
2.20.4 Turkey 30
2.20.5 Iran 31
2.20.6 China 31
CHAPTER THREE: MATERIALS AND METHODS **32**
3.1 Study Area **32**
3.2 Study Design **34**
3.3 Sample Size Determination **34**
3.4 Sample Collection and Procedure **35**

3.4.1 Administration of Structured Questionnaires 36

3.5 Sample Processing..... 36

3.5.1 Examination of dog faeces for *L. serrata* eggs..... 37

3.5.2 Examination of nasal swab from dog for *L. serrata* eggs..... 37

3.5.3 Determination of infestation of slaughtered dogs with adult *L. serrata*..... 38

3.6 Data Analysis..... 38

CHAPTER FOUR: RESULTS..... 40

4.1 Prevalence of *Linguatula serrata* in Slaughtered Dogs in Zaria and Environ..... 40

4.1.1 Prevalence of *Linguatula serrata* in nasal cavity of slaughtered dogs..... 40

4.1.2 Prevalence of *Linguatula serrata* in tongue of slaughtered dogs..... 40

4.2 Prevalence of *Linguatula serrata* eggs in Faeces and Nasal Secretions of Household Dogs..... 41

4.2.1 Presence of *Linguatula serrata* eggs in faeces of household dogs..... 41

4.2.2 Presence of *Linguatula serrata* eggs in nasal secretion of household dogs..... 41

4.2.3 Prevalence of *Linguatula serrata* in nasal secretion based on sex of dogs..... 47

4.2.4 Prevalence of *Linguatula serrata* in nasal secretion based on age of dogs..... 47

4.2.5 Prevalence of *Linguatula serrata* in nasal secretion based on the breed of dogs..... 47

4.2.6 Prevalence of Linguatula serrata based on purpose of keeping dogs…….. 51

4.3 Results of Assessment of Risk Factors for *Linguatula serrata* Infection in Household Dogs..... 53

4.3.1 Demographic features of the Respondents..... 53

4.3.2 Other informations on dogs from respondents..... 53

4.3.3 Prevalence due to risk factors that may predispose to *Linguatula serrata* infection in dogs……..54

4.3.4 Prevalence of *Linguatula serrata* based on wards sampled..... 57

4.4 Other Parasites Found in Faeces of Household Dog(s)…… 57

CHAPTER FIVE: DISCUSSION..... 60

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS..... 67

6.1 Conclusion 67

6.2 Recommendations 68

REFERENCES 69

LIST OF TABLES

Table	Title	Page
4.1	Prevalence of *Linguatula serrata* in household dogs in Zaria and environ…….	46
4.2	Prevalence of *Linguatula serrata* in nasal secretion based on sex of dogs in Zaria and environ……	48
4.3	Prevalence of *Linguatula serrata* in nasal secretion across age groups of dogs……..	49
4.4	Prevalence of *Linguatula serrata* in nasal secretion based on breed of dogs……..	50
4.5	Prevalence of *Linguatula serrata* in nasal secretion based on the purpose dog's are kept…….	52
4.6	Demographic characteristics of respondents (dog owners).....……..	55
4.7	Occurrence of risk factors that may predispose to *Linguatula serrata* infection in dogs …….	56
4.8	Prevalence of *Linguatula serrata* eggs in nasal secretion of dogs based on wards……	58
4.9	Other parasites found in faeces of dogs in Zaria and environ……	59

LIST OF PLATES

Plate	Title	Page
I	Head of a slaughtered dog split longitudinally showing nasal and other cavities of the head…….	42
II	Tongue of dog dissected ventrally showing lyssa fibrous connective tissue……	43
III	Histopathological section of the tissue material purported to be *L. serrata* in the ventrum of the tongue (Haematoxylin and eosin, X400 magnification)……...……..	44
IV	Matured egg (ova) of *L. serrata* recovered from the nasal secretion of dog…… …….	45

LIST OF FIGURES

Figure	Title	Page
2.1	Life cycle of *Linguatula serrata* and definitive hosts…….	13
3.1	Zaria Urban Area showing the Sampling Points…….	33

LIST OF APPENDICES

Appendix	Title	Page
I	Research questionnaire on risk factors that may predispose to *L. serrata* infection in dogs…….	80
II	Reference photographs for the confirmation of adult parasite and egg of *Linguatula serrata*……	83

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Linguatula serrata species belongs to the Pentastomida, a still-enigmatic group of worm-like, bloodsucking parasites that inhabit the upper respiratory tract of terrestrial, carnivorous vertebrates, mostly reptiles and birds. *Linguatula serrata*, commonly called "tongue worms", typically inhabits canids and felids. The intermediate hosts of these parasites are usually sheep, cattle, camels or rodents. The hosts ingest the eggs, and the first larva instar hatches within their intestines, penetrates the mucosa, and retreats into the tissue, where it encysts and molts to the third larval stage. Humans can serve as aberrant final hosts after ingesting raw or poorly cooked viscera (i.e., liver, lungs, and trachea) of intermediate hosts. This nasopharyngeal infection is known as Halzoun syndrome in the Middle East or as Marrara in Sudan (Yagi *et al*., 1996; Siavashi *et al*., 2002). Humans can also serve as accidental intermediate hosts, when ingesting the eggs (visceral infection) (Tappe and Butter, 2009). Intraocular infection has been reported but is extremely rare; only 5 cases caused by *L. serrata* tongue worms have been described: two from the Southern United States (Hunter and Higgins, 1960; Deweese *et al*., 1962), one from Portugal (Sousaefaro and Pinhao, 1964), 1 from Israel (Lang *et al*., 1987), and one from Ecuador (Lazo *et al*., 1999).

The phylogenetic position of the pentastomids is still not fully resolved. A position as the sister group of branchiurian crustaceans (Maxillopoda) is supported by molecular data and sperm morphology (Wingstrand, 1972; Lavrov *et al*., 2004; Møller *et al*., 2008) and is

widely accepted today; however, sound evidence also exists for other classifications (Almeida *et al*., 2008). The group itself is divided into 2 orders, the more primitive Cephalobaenida and the more advanced Porocephalida. All species infecting humans are currently classified as Porocephalida; the species *L. serrata* and *Armillifer armillatus* are responsible for most human cases of infection.

Linguatulids reside primarily in the respiratory ducts of carnivorous reptiles, birds, and mammals (John and Petri, 2006). The human infection is often contracted through ingestion of contaminated food and may manifest as nasopharyngeal, visceral, or ocular forms (Lazo *et al*., 1999; Siavashi *et al*., 2002). *L. serrata* commonly resides within the nasal passages of canines and felines and occasionally humans. The symptoms in humans are nasopharyngitis, violent coughing, asphyxiation, edematous congestion of gums, tonsils and Eustachian tube, aural pruritus, deafness, frontal headache, sneezing, lacrimation, coryza, yellow nasal discharge, facial edema, vomiting and breathing blockage (Muller, 2002; Mehlhorn, 2004; Schlossberg, 2004).

The life cycle of linguatulids involves two hosts. Development of larva occurs in an intermediate host, which begins by ingesting the eggs present in sputum, faeces, or body cavity of definitive hosts. Female worms produce several million eggs with the size of 90-70μm. The eggs, containing fully-grown larvae, are discharged with nasal secretion of definitive hosts including carnivorous, reptiles, birds and mammals into water or on vegetables. Once the eggs are ingested by intermediate hosts (e.g., fish, cattle, sheep, rabbits, rodents, ungulates) they hatch in the intestine and release the larvae, which then

burrow through the intestinal wall and lodge in liver, lungs and other viscera. Within the viscera, the larva matures to a pupa-like stage, and then to a nymph (infective larva) stage in which hooks, annular rings, and spines develop. After maturation to the nymph stage, the infective larva migrates to the pleural cavity. Once the intermediate host is eaten by a natural definitive host, the larvae migrate to the nasal cavities, where they mature into adults. Humans may serve both as intermediate host with encapsulated larvae in inner organs, and definitive hosts with adult worms in nose (Ma *et al*., 2002). Human infections can result from ingestion of raw or undercooked visceral tissues of the intermediate hosts such as sheep, goats, cattle, buffaloes or other herbivores harboring the larval stages of the parasite. The infection can also occur through drinking of water or ingestion of fruits and vegetables contaminated with *Linguatula* eggs. Halzoun Syndrome (Middle East) or Marrara syndrome (Sudan) are the human diseases described as pentastomids infection of man (Mehlhorn, 2004).

Linguatula serrata can be found worldwide but especially in warm subtropical and temperate regions. Human infection with *L. serrata* has been reported from different parts of the world including tropical regions of North and South America, Europe, Asia, Africa and Australia (Riley *et al*., 1985; Cheng, 1986). There are also some reports of the infection from different regions of Iran (Arbabi *et al*., 1996; Yeganeh *et al*., 2001; Maleky, 2001; Siavashi *et al*., 2002; Meshgi and Asgarian, 2003; Anaraki *et al*., 2008). The parasite is the most commonly reported pentastomid parasite of dogs and it corresponds to over 99% of reports from pentastomid infection (Lazo *et al*., 1999).

Linguatulosis often goes undetected, but can be diagnosed by mechanically abstracting and microscopically identifying linguatulids from the larynx, nose, and gums, or linguatulid eggs from nasal mucus. *Linguatula serrata* nymphs are commonly identified by their rows of spines, and anterior extremity bearing four hooks and a mouth (Siavashi *et al*., 2002; Mehlhorn, 2004). Nymphs migrate to the liver, spleen, lungs, and mesenteries where they encyst and calcify making them visible on chest or abdominal radiographs (Cheng, 1986; John and Petri, 2006).

Because Pentastomiasis is mostly asymptomatic, it's often not detected until autopsy. X-rays often reveal calcified nymphs, crescent-shaped bodies, distributed throughout the body. Because Pentastomes do not trigger massive specific immune reactions, serologic tests are harder to perform, although one has been developed and is in use in France. The test involves using gel immunodiffusion and indirect immunoflourescence, and requires high amounts of antibody (Drabek, 1987).

1.2 Statement of Research Problem

Linguatula serrata is a cosmopolitan species and both larval and nymphal stages have been recorded from humans in Africa, Europe, and the Americas (Beaver *et al*., 1984). *Linguatula serrata* has been reported to be present in some states of Nigeria in dogs. A prevalence of 38.57% and 35.67% was recorded in 2010 and 2011, respectively, in Jalingo, Taraba State in dogs (Oseni *et al*., 2014). An overall prevalence of 1.3% in food animals was reported by Nassouradine (2014) with 3% and 1.7% prevalence camels and goats

respectively. The study also found prevalence of 2%, 1% and 2% in Kaduna, Kano and Zaria respectively.

The intermediate hosts (cattle, sheep, goat, etc) are mostly found in the same environment with the definitive host (dog). Oseni *et al*. (2014) also reported that about one-third of dogs were found to be infected with *L. serrata* and the close contact between dogs and livestock may be responsible for the greater rates of infection since humans get infected through the consumption of raw or poorly cooked meat. The parasite was also reported from Nigerian royal python, though a different species *Armillifer* spp (pentastomid), which may be an alert of the possibility of an on-going zoonotic transmission of pentastomiasis from snake to man, (Ayinmode *et al*., 2010).

There is paucity of data on the prevalence of *L. serrata* parasite in dogs in the study area, and even the North western zone of the country. *L. serrata* in canines and humans can also lead to nasopharyngeal linguatulosis. Deaths have been reported in humans due to blocked air passages (Bowman *et al*., 2004).

Eggs, particularly those expelled from infected dogs by sneezing or in nasal secretions, are easily unwittingly ingested as contaminants of food, fingers, water, and fomites. In Nigeria, risk factors such as eating undercooked or poorly roasted meat, chicken or viscera and poor hygiene may pose danger of infection to humans. The symptoms of the disease in humans have similar signs with other endemic bacterial and viral conditions that causes sore throat and pharyngitis and for these reasons it is highly probable that the disease is misdiagnosed.

1.3 Justification

Dogs are generally easily infected with this parasite and are the major source of linguatulosis of herbivorous animals and man. Because of lack of information on the prevalence of linguatulosis of dogs in the Northern areas of Nigeria, determining the frequency of infestation of this disease in dogs from the public health point of view is of great significance. Therefore, the result obtained from this work will establish the prevalence of *L. serrata* in slaughtered and household dogs in Zaria metropolis since there is paucity of information on the prevalence of the parasite in dogs in the study area.

Close contact to dogs and their secretions predispose to infection with *L. serrata* (Tappe and Butter, 2009). The highest prevalence of visceral linguatulosis due to *L. serrata* has been reported from the Middle East, where high infection rates for dogs have also been noted. Studies have shown that *L. serrata* was found in 43.3% of stray dogs in Beirut, Lebanon, 38% in parts of India, and in a high percentage in Mexico City (Acha and Szyfers, 2003). Some locals also believe that eating the raw or undercooked offals, especially liver of farm animals (cattle or sheep), is a useful means of promoting fetal growth during pregnancy because of its high content of iron and vitamins (Oseni *et al*., 2014). There have also been reported cases of some Nigerians and Congolese emigrants to Europe and North America that died and were diagnosed with pentastomiasis (Tappe, 2013).

The result obtained from this research will serve as baseline information for the disease control and surveillance which may be used for the sensitization of human and animal health workers on the disease and its importance in the area. In addition, the work will

establish a prevalence of *L. serrata,* which is important in managing patients as the disease is not treatable with antibiotics for better medical care, and necessary treatment, which will help to reduce human suffering due to wrong diagnosis and inappropriate treatment.

1.4 Aim and Objectives

1.4.1 Aim

To determine the prevalence and risk factors associated to *L. serrata* infection in dogs in Zaria and environs, Kaduna State.

1.4.2 Objectives

The objectives were to:

1. determine the prevalence of *L. serrata* in slaughtered dogs in Zaria and environ.
2. determine the prevalence of *L. serrata* eggs (ova) in faeces and nasal secretion of household dogs in Zaria and environ.
3. determine the risk factors that may predispose to *L. serrata* infection in household dogs in Zaria and environ.

1.5 Research Questions

1. Is *L. serrata* prevalent in slaughtered dogs in Zaria and environ?
2. Is *L. serrata* eggs (ova) present in faeces and nasal secretions of household dogs in Zaria and environ?

3. What are the risk factors that may predispose to *L. serrata* infection in household dogs in Zaria and environ?

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of Pentestomids

The phylum pentastomida consists of about 100 species of endoparasites of the respiratory tract of vertebrates. Of importance in veterinary and human medicine are the two families Linguatulidae and Procephalidae of which species of the genus Linguatula and Armillifer are zoonotic parasites (Schmidt and Roberts, 1989). *Linguatula serrata* is commonly referred to as tongue worm, because many species within the phylum have transparent tongue-shaped bodies (Blagburn *et al*., 1983). The life cycle of *Linguatula* sp. involves two hosts. Dogs, cats, foxes and other carnivores are the definitive hosts of this cosmopolitan parasite. Transmission to intermediate hosts occurs through nasal secretions. Intermediate hosts include human beings and other mammals, but are often herbivores such as cattle, goats, sheep and other ruminants that have ingested plants contaminated with parasite eggs. Both adults and nymphs of *L. serrata* may parasitize human beings (Besch, 1959; Fain, 1975). Usually, because of the absence of specific clinical symptoms in parasitized dogs, diagnosis of linguatulosis is difficult.

Linguatulosis is a rare zoonotic parasitic disease resulting from invasion of the body by wormlike parasites of the genus *Linguatula*, also known as tongue worms (Muller, 2002). The most commonly reported species involved in Linguatulosis is *Linguatula serrata* (family Linguatulidae, order Porocephalida, and phylum Pentastomida), which is commonly classified between annelids and arthropods (Mehlhorn, 2004).

The species *Linguatula serrata* are enigmatic group of aberrant, worm-like, bloodsucking, zoonotic, and obligate endoparasites (Gosling, 2005; Fard *et al*., 2010a) that inhabit the upper respiratory tract of terrestrial, carnivorous vertebrates.

The adult form of this parasite inhabits the nasal airway, frontal sinus, and tympanic cavity of canids and felids (Alcala- canto *et al*., 2007). The intermediate hosts of these parasites are usually sheep, cattle, or rodents in which visceral linguatulosis have been described. The parasite is the most commonly reported pentastomid parasite of dogs and it corresponds to over 99% of reports from pentastomid infection (Lazo *et al*., 1999). In most cases, the parasites were detected at surgery or at autopsy, mainly in the liver, lungs, and lymph nodes. Diagnosis of linguatulosis is often difficult because of the absence of specific clinical symptoms in parasitized dogs.

2.2 Morphology of Pentestomids

Most adult linguatulids are elongate, worm-like, cylindrical, and tongue-shaped. Some adult species have body annulations or rings. The anterior extremity of linguatulids is characterized by a mouth, four hooks, and broad tongue-shape. The posterior extremity is often narrow and cylindrical (Sambon, 1922; John and Petri, 2006).

Nymphs

Nymphs are C shaped, contain hooks and spines, are less than 1 cm in diameter, and can calcify, allowing visibility on chest or abdomen radiographs (Ma *et al*., 2002; John and Petri, 2006).

Linguatula serrata

Linguatula serrata females are 80-130 mm long; 8-10 mm wide anteriorly, 2 mm posteriorly. Males are 18-20 mm long; 3-4 mm wide anteriorly, 0.5 mm posteriorly. Eggs are 90 by 70 microns. Color is yellowish and nymphal forms have a single row of minute spines (Sambon, 1922).

2.3 Reproduction

Although sexually dimorphic, the larger female attempt to find similarly sized male to mate. *Linguatula serrata* females mate only once over the course of their lifetime, but due to huge spermathecal storage and oocytes continuously being ejected from the ovary; female can lay thousands or millions of eggs per year (Riley *et al*., 1985)

2.4 Life Cycle of *Linguatula serrata*

The life cycle of linguatulids involves two hosts. Larval development occurs in an intermediate host, which ingests eggs contained in the sputum, faeces, or body cavity of definitive hosts (Hobmaier and Hobmaier, 1940; Cheng, 1986; John and Petri, 2006). Eggs (90-70 microns) contain fully formed larvae. Females produce many eggs, several million, which are passed from the nasal discharge of definitive hosts (e.g., carnivorous reptiles, birds, mammals) to water or vegetation. Intermediate hosts (e.g., fish, cattle, sheep, rabbits, rodents, ungulates) then ingest infected water or vegetation. Eggs hatch in the intestine of intermediate hosts releasing the larvae, which then burrow through the intestinal wall and lodge themselves in the liver, lungs and other viscera. Within the viscera, the larvae mature to a pupa-like stage, then to a nymph (infective larva) stage in which hooks, annular rings,

and spines develop. After maturation to the nymphal stage, the infective larva migrates to the pleural cavity (Hobmaier and Hobmaier, 1940; Cheng, 1986; John and Petri, 2006).

2.4.1 Definitive host of *Linguatula serrata*

If the intermediate host (e.g., a fish, cattle, sheep, rabbit, rodent, ungulate) is then eaten by a natural definitive host (e.g., a carnivorous reptile, bird, mammal) the infective larva migrate to the nasal cavities where they mature into adults. If the intermediate host is not eaten by a natural definitive host, there is no observed migration to the nasal cavity. Rather, the infective larvae migrate to the intestines, penetrate, and exist within the body cavity (Hobmaier and Hobmaier, 1940; Cheng, 1986; John and Petri, 2006). Although cases of linguatulosis in humans is rare, humans may serve as both intermediate and definitive hosts upon ingesting infective eggs and infective nymphs, respectively for linguatulids (Ma *et al*., 2002).

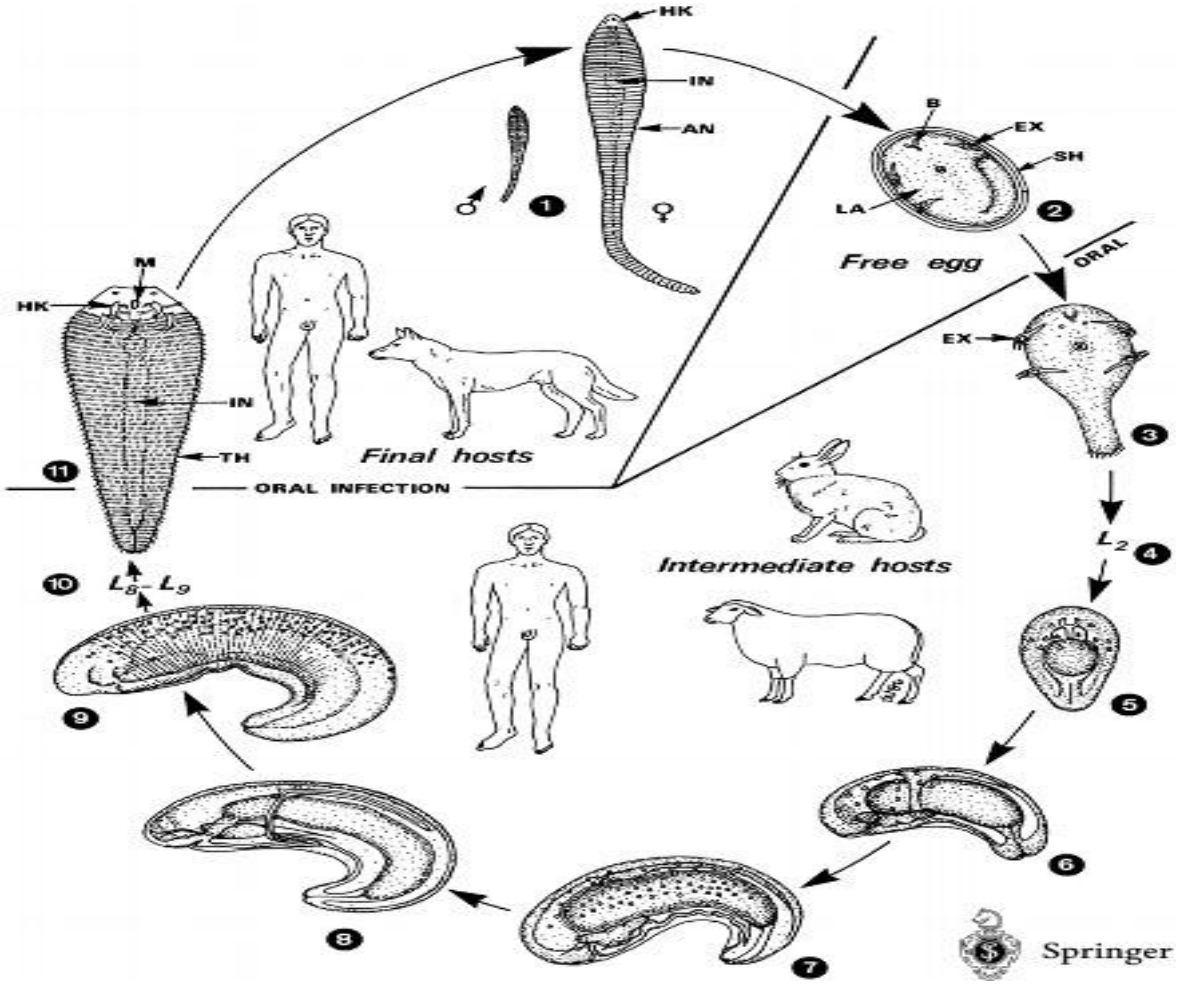


Figure 2.1: Life cycle of *Linguatula serrata* (1) Adults live in the nose of dogs (and rarely of man). (2) Embryonated eggs are set free via nasal mucus and/or feces. The thin outer is left out in drawings, since it disappears soon. (3) If intermediate hosts swallow eggs, the four-legged primary larva hatches and migrates via blood vessels to the inner organs. Humans may also become accidental intermediate hosts. (4-11) Larval stages 2–11 are included in a capsule of host origin and grow after molts. When final hosts ingest raw (or uncooked) meat of intermediate hosts, the adult stages develop inside the nasal tract. Infected humans suffer from the AN, annuli; B, EX, extremity with a claw; MK, mouth hooks; IN, intestine; LA, primary larva; M, mouth; SH, inner eggshell; TH, thorns" (Mehlhorn, 2004).

2.5 Incubation Period of *L. serrata*

The time of larval stage development varies across species; however, it generally takes only a short amount of time (several hours) for ingested Pentastomid eggs to hatch and become primary larvae. The larvae may then remain encysted for weeks or months (Hopps *et al*., 1971). In the case of Halzoun syndrome, the third-stage larvae migrate almost immediately to the nasopharyngeal tract and begin to cause symptoms within a few hours.

2.6 History of *Linguatula serrata*

Pentastomiasis as an animal infection was first described in 1787 by the French veterinary surgeon Chabert Froelich, who found *L. serrata* in the nasal cavities of several dogs and horses (Cannon, 1942). The taxonomy of the Pentastomids has always been problematic: In 1819, the *Linguatula* and several other similar genera were grouped together under the name Pentastomum. In 1848, Van Beneden placed it under genus Arthropoda, and Leuckart in 1860 under the class Arachnida. The Pentastomids were considered Arthropods until the middle of the twentieth century, when it was decided that they should be considered a separate phylum.

The earliest classification of the genus *Linguatula* was in 1789 by Froelich. Numerous *Linguatula*-related classifications occurred between 1789 and the early 1900s. These include classification of *Linguatula serrata* in 1789 by Froelich, and Linguatulina, a separate order for Tongue-worms, by Hayck in 1881 (Sambon, 1922).

The first human infection by a pentastomid was described by Pruner in Cairo in 1847 (Cannon, 1942). Early in its history, Pentastomiasis received much attention from

prominent pathologists, but subsequently its study became more sporadic. Leuckart in the 1860's, Sambon in the 1920's, and Fain in the 1960's contributed greatly to our knowledge of Pentastomiasis. Several monographic articles have been written about Pentastomiasis since then, but it still remains a rather obscure condition.

2.7 Epidemiology

Linguatula serrata, the most common cause of linguatulosis in humans, has been reported in the tropical regions of North and South America, Europe, Asia, Africa, and Australia. It is most common in Malaysia and Central Africa (John and Petri, 2006).

Human cases of visceral linguatulosis have been linked with eating raw visceral organs of sheep, goats, cattle, or camels in India, Turkey, Greece, Morocco, and Sudan. Linkages with eating undercooked sheep, goat liver, or lymph nodes has been reported in Lebanon and Iran (Riley *et al*., 1985; Cheng, 1986; Lazo, 1999; Ma *et al.,* 2002; Siavashi *et al.*, 2002; Meshgi and Asgarian, 2003; John and Petri, 2006). Two human cases of ocular linguatulosis were reported in the USA, one in Israel and one in Ecuador (Lazo *et al*., 1999).

Linguatulosis in humans has been reported from Africa, Southeast Asia, America and the Middle East (Gardiner *et al*., 1984; Baird *et al*., 1988; El-Hassan *et al*., 1991; Yagi *et al*., 1996; Lazo *et al*., 1999; Acha and Szyfres, 2003). Several reports have documented nasopharyngeal linguatulosis in Iran following consumption of liver Kebab (barbecue) (Hodjati and Naghili, 1987; Fata *et al*., 1994; Sadjjadi *et al*., 1998). A 28-year-old woman affected with linguatulosis and with respiratory symptoms, including sneezing and nasal

discharge, representing the clinical signs of Marrara was described in Iran (Maleky, 2001). Human nasopharyngel linguatulosis was also reported from the Van Province of Turkey close to the North Western region of Iran (Yilmaz *et al*., 2011).

Several other studies have assessed the prevalence of *L. serrata* in dogs (Khalil, 1970; Akyol *et al*., 1995; Oryan *et al*., 1997; Meshgi and Asgarian, 2003; Aldemir, 2004; Oryan *et al*., 2008), one-humped camels (Mobedi *et al*., 1988; Oryan *et al*., 1993; Tajik *et al*., 2007; Haddadzadeh *et al*., 2010; Radfar *et al*., 2010), two-humped camels (Haddadzadeh *et al*., 2009), buffaloes (Sisakumar *et al.,* 2005; Tajik *et al*., 2008; Tajik and Jalali, 2010), sheep (Esmail-Nia *et al*., 2000; Shekarforoush *et al*., 2004; Tavassoli *et al*., 2007a; Ravindran *et al*., 2008; Gul *et al*., 2009; Fard *et al*., 2011), cattle (Tajik *et al*., 2006; Hami *et al*., 2009; Fard *et al*., 2010b; Youssef and Hadizedeh-Moalem, 2010), goats (Saiyari *et al*., 1996; Razavi *et al.,* 2004; Tavassoli *et al*., 2007b; Mir *et al*., 2009; Fard *et al*., 2010a;) and small ruminants (Vatsya *et al*., 2011). In addition, adult and nymph stages of the parasite have been isolated from red foxes (Gicik *et al*., 2009).

2.8 Risk Factors for Infection

Close contact to dogs and their secretions predispose to infection with *L. serrata*, whereas people whose diet includes snake meat, workers at Asian snake-farms, snake keepers in zoos and pet shops, veterinarians, and owners of several species of pythons, vipers, cobras, and rattlesnakes may be exposed to ova of *Armillifer* and *Porocephalus*.

In Nigeria, risk factors such as eating undercooked or poorly roasted meat and viscerals, poor hygiene and vegetative contamination of herbs with dog's faeces may also pose

danger to humans. Consumption of dog meat and offals as delicacies by some tribes in Nigeria may also be a predisposing factor to this zoonoses (Tappe and Butter, 2009).

2.9 Pentastomiasis in Nigeria

In the mid- eastern part of the country, *Armillifer armillatus* has been reported by (Azinge *et al*., 1978). Nwosu *et al*. 2002 reported retrospective and prospective studies, for prevalence of *Linguatula serrata* among dogs, around the Mambilla plateau, North- eastern Nigeria, the retrospective covered a ten year period (1990-1999) at the state veterinary clinic, out of 1439 dogs examined, 1034 (71.9%) were infected with the *L. serrata*. While the 29.4% of 218 dogs examined during the prospective study were infected (Nwosu *et al*., 2002). In the same state in Jalingo prevalence of 37.45% was recorded by (Oseni *et al*., 2014). *Armillifer* species have been reported in a 57 year- old female Nigerian farmer- a habitual snake eater- presented with clinical and radiological features of Armillifer infestation with hepatic encephalophathy (Ogun, 2011).

In most cases, the parasites were detected at surgery or at autopsy, mainly in the liver, lungs, and lymph nodes not under diagnostic condition this may be due to absence of specific clinical symptoms in parasitized dogs (Tappe and Butter, 2009).

2.10 Predilection Site for the Parasite

Pentastomid nymphs may be found throughout the peritoneal cavity, from which motile living parasites can be extracted during surgery or autopsy. Most parasites are located in the subperitoneal tissue around the liver, mesentery, spleen, and in the intestinal wall (Reeder *et al*., 2000). Infections of the liver parenchyma (Gast- Galvis, 1960; Arroyo *et al*., 1986;

Baird *et al*., 1988; Ma *et al*., 2002; Machado *et al.*, 2006) and abdominal lymph nodes (Arroyo *et al*., 1986; Ma *et al*., 2002) are also frequently noted. Less often, parasites are found in the parenchyma of the lungs and on the pleural surface (Pampiglione *et al*., 2001; Tappe *et al*., 2006). Rarely, involvement of the heart (Ma *et al*., 2002), eye (*Lazo et al.*, 1999), and other organs has been described.

2.11 Clinical Features and Pathogenesis

Human infections can result from ingesting of raw or undercooked visceral tissues of the intermediate hosts such as sheep, goats, cattle, buffaloes or other herbivores harboring the larval stages of the parasite. The infection can also occur through drinking of water or ingestion of fruits and vegetables contaminated with *Linguatula* eggs. Halzoun Syndrome (Middle East) or Marrara syndrome (Sudan) are the human diseases described as pentastomids infection of men (Mehlhorn, 2004). Human infection with *L. serrata* has been reported from different parts of the world including tropical regions of North and South America, Europe, Asia, Africa and Australia (Riley *et al*., 1985; Cheng, 1986). There are also some reports of the infection from different regions of Iran (Arbabi *et al*., 1996; Maleky, 2001; Yeganeh *et al*., 2001; Siavashi *et al*., 2002; Meshgi and Asgarian, 2003; Anaraki *et al*., 2008).

The human infection is often contracted through ingestion of contaminated food and may manifest as nasopharyngeal, visceral, or ocular forms (Lazo *et al*., 1999; Siavashi *et al*., 2002). *L. serrata* commonly resides within the nasal passages of canines and felines and occasionally humans. The symptoms in humans are nasopharyngitis, violent coughing, asphyxiation, edematous congestion of gums, tonsils and Eustachian tube, aural pruritus,

deafness, frontal headache, sneezing, lacrimation, coryza, yellow nasal discharge, facial edema, vomiting and breathing blockage (Mehlhorn, 2004; Schlossberg, 2004; John and Petri, 2006).

Humans are usually tolerant to nymphal pentastomid infections, and most patients are asymptomatic. The living nymph provokes little inflammation, whereas the death of the parasite leads to a prominent host response (Baird and Carey, 1997). Most findings of visceral linguatuliasis are made at autopsy (Tappe *et al.,* 2006), and the parasites are mainly located in the liver. Infection of the lung is rare (Koch, 1906). The nymphs in human granulomas are typically degenerated at the time of examination (Baird *et al*., 1988; Lazo *et al*., 1999), but the cuticle with its associated structures remains visible for some time (Baird and Carey, 1997).

The initial phase of pentastomiasis in all intermediate hosts is probably the penetration of the gut wall by first- stage larvae, after embroyonated eggs have been ingested in contaminated meat, water, or vegetation (Wayne and Ronald, 2011). In the abdomen, first-stage larvae molt twice and develop into infective third- stage larvae. Most, but not all, third- stage larvae encyst and lodge in various sites, showing a predilection for the mesenteric and peritoneum, and on or within parenchymatous abdominal organs. First-stage larvae that enter the lymphatic or blood system in the gut wall frequently invade the lungs and occasionally the brain, but may appear in virtually any site. Even when infection involves numerous third- stage larvae, pentastomiasis is usually asymptomatic. However, as the developing larvae gradually enlarge, they may exert pressure on surrounding structures, causing complications such as biliary obstruction, dysphagia and dyspnea

(Wayne and Ronald, 2011). In the eye, pentastomes may cause multiple lesions, including iritis, subluxation of the lens, secondary glaucoma, conjunctivitis, and invasion of lacrimal caruncles (Lazar and Traub, 1967; Polderman and Manschot, 1979; and Lang *et al*., 1987). Other conditions attributed to pentastomes include, pneumonitis, collapsed lung, peritonitis, nepritis, meningitis, and pericarditis, in most cases only a tenuous casual relationship has been established (Tiendrobeogo *et al*., 1982; and Yapo *et al*., 2003). Intestinal obstruction and hepatoma like syndrome may be caused by pentastomiasis as well (Fisch *et al*., 1985; Machado *et al*., 2006)

2.12 Transmission

The epidemiology of *L. serrata* infections in man is complicated because both eggs and infective larvae can become established. Eggs hatch in the alimentary tract and primary larvae subsequently invade the body cavity to encyst on the viscera, producing Visceral Linguatulosis, whereas ingested infective larvae attempt to migrate to the nasal passages, producing Nasopharyngeal Linguatulosis (Khalil and Schacher, 1965).

2.12.1 Visceral linguatuliasis

In areas, where *L. serrata* is endemic, such as in the Middle East, Visceral Linguatulosis is probably much more common than is generally realized (Khalil and Schacher, 1965). Eggs, particularly those expelled from infected dogs by sneezing or in nasal secretions, are easily unwittingly ingested as contaminants of food, fingers, water, and formites; hence, veterinarians, dog handlers, and owners could be at risk of the infection and are better advised to wash hands after handling or treating dogs regularly.

In humans who accidentally serve as intermediate pentastome hosts, the infection develops when parasite ova are ingested from respiratory secretions or feces from the final hosts (dogs and some other carnivores for *Linguatula*, several species of large snakes for *Armillifer* and *Porocephalus*).

2.12.2 Nasopharyngeal linguatuliasis

Humans could also be infected with the larvae of *L. serrata* by eating raw glands of cattle, sheep, and goats that have the larvae. The nasopharyngeal form is also known as 'Halzoun syndrome' (named for the traditional Middle eastern dish made by raw mutton or beef) or "Marrara syndrome" (named for the traditional dish consisting of raw sheep or goat liver common People may suffer from irritation in their nose and throat. Deaths have been reported due to blocked air passages (Bowman *et al*., 2004).

Infection occurs when humans ingest raw or undercooked viscera (e.g., sheep, goat, or even camel liver) containing encapsulated infective nymphs. Most *Armillifer* infections are acquired when undercooked snake meat is consumed, but may also involve ingestion of water contaminated with snake feces. Humans become accidental intermediate hosts. Infective nymphs exit the digestive tract and develop in the liver, mesentery, and intestinal wall of the human host. Humans are dead-end hosts and nymphs, even if long-lived, eventually die and calcify (Paré, 2008). According to Tappe and Büttner (2009), in asymptomatic patients no treatment is necessary, since the parasites degenerate after approximately two years. Only in symptomatic infections with numerous parasites may a surgical approach have to be considered. There is no antiparasitic chemotherapy available for pentastomiasis. (Tappe and Büttner 2009).

2.13 Symptoms in Man

Although infection is usually asymptomatic, patients infected with linguatulosis can present with discomfort and a prickling sensation in the throat extending to the ears and nasal passages, which can occur as soon as thirty minutes after eating infected meat (Cheng, 1986). These symptoms can progress to frontal headache, Eustachian tube congestion, abscess in the auditory canal, facial swelling, and abdominal pain, with clinical signs such as mesenteric lymph node inflammation, jaundice, dyspnea, dysphagia, coughing, sneezing, yellow nasal discharge, and in rare cases ocular lesions (Cheng, 1986; Siavashi *et al*., 2002; John and Petri, 2006). Symptoms appear a few minutes after the infective food is consumed (Acha and Szyfres, 2003). Incubations period varies depending on where the nymphs are released from their cysts. The most common symptoms are throat irritation and pain. Sometimes there is congestion and intense edema of the area. Lacrimation and nasal discharge are common. At times, there is also dyspnea, dysphagia, vomiting headaches, photophobia, and exophthalmia. The course of the disease is rapid and benign. About half of patients recover in less than one day; in others, the illness may last one to two weeks (David *et al*., 2006; Schimidt *et al*., 2013).

Symptoms depends on the organ system involved and results from the death of the nymphs or their migration. If only a few parasites are present, *L. serrata* may mimic hepatic or pulmonary malignancy clinically and on radiological assessments. Patients may develop abdominal pain, chronic cough, or night sweats (Pampigliones *et al.,* 2001; Machado *et al*., 2006; and Tappe *et al*., 2006). In heavy infections with *Armillifer* spp, death may occur due to secondary septicemia, pneumonia, or severe enterocolitis (Ma *et al*., 2002; Yapo *et al*.,

2003). However, most human infections are asymptomatic, and the disease may be an incidental finding during routine medical consultation or at autopsy.

The symptoms usually subside within 7-10 days, but sometimes complications can occur. Damage to facial nerves or perforation of the eustacean tube has sometimes been observed. Halzoun syndrome is also related to infections of *Fasiola hepatica*, liver flukes (Drabick, 1987). The larvae, in almost every case ever reported, do not mature, their human host eliminating them within three weeks. However, in two reported cases, adult Linguatula developed within human's nasopharyngeal tracts, causing nosebleeds and pressure within the nose (Hopps *et al*.,1971). Infections of other areas, like the eyes, by third stage larvae, have also been observed.

In most cases infected dogs and cats show no clinical signs. However, massive infections can cause rhinitis (inflammation of the nose) and nasopharingitis with chronic sneezing and/or coughing, purulent nasal discharge, nose bleeding (epistaxis), and so on.

In livestock, most infections with nymphs are benign and remain asymptomatic. In case of infrequent massive infections, clinical signs may develop depending on the affected organs (Junquera, 2014).

2.14 Pentestomiasis and Cancer

The living nymph provokes little inflammation, whereas the death of the parasite leads to a prominent host response (Baird and Carey, 1997). Most findings of visceral linguatuliasis are made at autopsy. Galil- Valerio (1921) reported a human case of fibrosarcoma of the nasal cavities invaded by an adult of *Linguatula serrata*. Tobie *et al*. (1957) reported a

tongue worm (*Linguatula serrata*) infestation in a patient with acute leukemia. They have observed a bronchial carcinoma in a Komodo varanus which was infected with *Sambonia lohrmanni*. This cancer has apparently originated from a villous papilloma of the bronchial mucosa (Fain, 1975). Several young larvae of this pentastomid were found inside the intestinal villi. Other larvae or nymphs were embedded in the tracheal mucosa, this host was also infected by adult form of the same pentastomid (Fain, 1960). Although, these observations are not sufficient to prove the carcinogenic action of larval pentastomids. In addition, the frequency of the primitive cancer of the liver in Africans could be in relation with the great prevalence of the nymphal pentastomosis of the human liver in Central Africa (Meyers *et al*., 1976). It should be necessary to select for that purpose animals that are especially susceptible to this kind of cancer (Fain, 1975). In addition, many researchers speculate that pentastomes provoke malignancies (Smith *et al*., 1975).

2.15 Diagnosis of *Linguatula serrata*

A radiological diagnosis is possible when calcified nymphs of *Armillifer* spp. and less often *L. serrata* are detected on pulmonal or abdominal radiographs, showing a horseshoe or C-shaped structure (Reeder *et al*., 2000). There may be a mild eosinophilia. A few serological studies have been conducted (Nozais *et al.*, 1982); however, no serological test is readily available, and no PCR test has been established for the diagnosis from biopsied tissues. Thus, visceral pentastomiasis often remains a histopathological diagnosis. The pentastome nymphs may be mistaken for other tissue-dwelling metazoan parasites, or, due to their peculiarity, the diagnosis may be delayed. The discrimination of pentastomes from other

metazoa is needed to treat the patients who have infections with nematodes, cestodes, or trematodes.

Diagnosis should be made etiopathologically, subetiopathologically, or presumptively on the basis of whether entire nymphs, cuticle-associated structures, or pearly lesions ("*Linguatula* nodules") (St Symmers and Valters, 1950) with targetoid appearance are found. The differential diagnosis includes malignancies and tuberculosis because of the radiologic coinlike appearance. On histologic examination, one must distinguish between tissue-inhabiting diptera larvae, infections with metacestodes, trematodes, tissue filariids, and gnathostomiasis. Once diagnosis is established, no treatment is necessary for the parasites will degenerate after some time, and no effective antiparasitic therapy exists.

The method for diagnosis of *L. serrata* infestation differs with the animal species serving as the definitive or intermediate host. Diagnosis in dogs and cats is based on the respiratory signs and can be confirmed through detection of eggs in the nasal discharge or in the feces. However, egg shedding is intermittent, which can lead to false negatives. Adult worms can be directly visualized in the nasopharynx through rhinoscopy or rhinotomy (Junquera, 2014).

2.16 Treatment

Pentastomiasis is only treated when it becomes a serious medical condition. In these cases, surgical removal of the larvae is performed. For Halzoun Syndrome, clearing of breathing passageway is sometimes necessary (Strickland, 1991).

In asymptomatic patients no treatment is necessary, since the parasites degenerate after approximately two years (Lazo *et al.*, 1999). Only in symptomatic infections with numerous parasites may a surgical approach have to be considered. There is no antiparasitic chemotherapy available for pentastomiasis.

Exploratory laparotomy may be carried out for patients with acute abdominal symptoms or those with radiologic evidence of pentastomiasis. Infected mammals, snakes, and captive monitor lizards have been successfully treated with ivermectin, sometimes combined

In heavily infected dogs or cats surgical removal is often the only alternative. There are virtually no veterinary medicines approved for the control of *Linguatula serrata* in dogs, cats or livestock. There are a few reports showing that injectable ivermectin was effective against a related species, *Linguatula arctica* in reindeer. However, treatment of cattle, sheep or other livestock with parasiticides against tongue worms is usually not indicated, because they cause no economic damage. There are so far no true vaccines against *Linguatula serrata* (Junquera, 2014).

2.17 Prevention and Control of *Linguatula serrata* Infection

The best preventative measure in endemic regions consists in keeping dogs and cats away from raw innards of cattle, sheep, and other livestock, and to scavenge on potentially contaminated food (e.g. abattoir scraps). This will also diminish their output of *Linguatula* eggs into the environment, which is the major source of contamination for livestock and humans (Junquera, 2014).

To prevent human infections in endemic regions the basic hygienic measures have to be followed: frequent hand washing with soap; never eat uncooked innards or vegetables; avoid too close contacts with pets and quickly remove their droppings, etc. (Junquera, 2014).

Larval infection through the ingestion of the eggs can be prevented by guarding against drinking water used by canine saliva and other wild carnivores or raw food contamination with depositions of the raw viscera of goats, sheep or other herbivores (Acha and Szyfres, 2003). Public health education on the route of the disease transmission to those at higher risk like dog handlers and snake owners should be conducted. There is need to establish endemic foci of the disease and its prevalence for policy making and stake holders sanitation (Baird *et al.,* 1988; Tappe and Butter, 2009).

There is need for improved meat inspection in *Linguatula serrata* in endemic regions to prevent human infection.

2.18 Economic Importance for Humans

Many human infections arise in underdeveloped areas of the world like the Middle East, India, Africa, and Southeast Asia, where eating raw glands of cattle, sheep, and other herbivores mammals is a part of normal diet. Infection may also be acquired trough ingestion of eggs from plants or water in the environment. Infestations of the worm are frequent in humans, and deaths have even been reported due to blocked nasal passages. Because human are often the accidental intermediate or final host of *L. serrata*, the pentastomid is of medical and economic importance (Behrmann, 2012). Those who can

afford medical help can direct their treatment either towards eliminating the nymphs in their system or relieving the symptoms of infestation through nasal sprays or antihistamines (Tavassoli *et al*., 2007a).

2.19 Distribution of Pentastomiasis

The highest prevalence of visceral pentastomiasis due to *L. serrata* has been reported from the Middle East (Khalil, 1972), where high infection rates of dogs, the final host for *Linguatula*, have been noted. In Central and South America, sporadic cases have also been described (Gast- Galvis, 1960; Arroyo *et al*., 1986; *Lazo et al.*, 1999; Machado *et al*., 2006). The disease is rare in Europe, the United States, and China, where only a few cases have been reported (Parenzan and Chieffi, 1951; Gardineer *et al*., 1984; Baird *et al*., 1988; Pampiglione *et al*., 2001; Ma *et al*., 2002; Tappe *et al*., 2006). Infections with *Armillifer* spp. are most prevalent in West Africa, Central Africa (Self *et al*, 1975; Smith *et al*., 1975; Nozais *et al*., 1982; Tiendrebeogo *et al*., 1982; du Plessis *et al*., 2007; Yapo *et al*., 2003; Dakubo *et al*., 2006), and Malaysia (Prathap *et al*., 1969), where snakes, the final hosts of these parasites, are locally prepared for food. Autopsies performed in Nigeria revealed that in 33% of patients who died of malignancies, *Armillifer* was found (Smith *et al*., 1975). A high frequency of 45% was also reported from a general autopsy study of aborigines in West Malaysia (Prathap *et al*., 1969). In Europe and North America the disease is rarely diagnosed, but it may be observed in immigrants from endemic areas and long-term travelers. The disease prevalence of 71% was reported from dogs at Mambilla Plateau state but data on human prevalence around the areas is limited. The more detailed distribution is indicated below:

2.20 Cases Reported

2.20.1 Australia

A 14-year-old girl was referred to the eye clinic at the Medical University of Vienna with an unknown parasite detected during ophthalmologic examination. The girl had redness, pain, and progressive visual loss in the right eye. Her medical history was unremarkable except that she had reported regular contact with domestic animals: 2 dogs, cats, and 1 turtle. She had no history of bites or other infestations, and neither she nor any of her animals had been abroad. Surgical removal of the parasite was complicated because of high mobility of the parasite inside the anterior chamber. The worm escaped into the posterior segment of the eye where it was found, after lens removal and complete vitrectomy, in a recess of the ciliary body. The surgically extracted parasite was examined microscopically and then subjected to DNA isolation (Koehsler *et al*., 2011).

2.20.2 Germany

A recent infection with *L. serrata* in Germany in a patient who had pulmonary symptoms and in whom malignancy was suspected was reported. The patient was a 39-year-old man of Russian origin who had been living in Germany since 1999. He was admitted to the hospital with weight loss, night sweats, chest pain, and coughing. He had been a smoker for 20 years, and his past medical history included pneumonia and sinusitis in 1989 during his military service at Lake Baikal, Russia. The patient had been living in a farmhouse in Karaganda, Kazakhstan, until he immigrated to Germany (Tappe *et al.,* 2006).

2.20.3 Ecuador

The patient studied was a 34-year old woman from Guayaquil, Ecuador who complained of ocular pain with conjunctivitis and visual difficulties of two- month duration. Biomicroscopic examination revealed a mobile body in the anterior chamber of the eye. The mobile body was surgically removed. The specimen was fixed in alcohol, cleared using the technique of Loos, stained with acetic carmine, and mounted on balsam between a slide and a cover slip. It was observed with stereoscopic and common light microscopes in combination with an automatic system for image analysis and processing. The morphologic and morphometric characteristics corresponded to the third-instar larval form of *L. serrata* (Lazo *et al*., 1999).

2.20.4 Turkey

The patient, who was 26 years old, lived in Erciş a town in Van province, East Turkey. She was admitted to the Outpatient Clinic of Infectious Diseases of Erciş Government Hospital with a complaint of coughing a few worms about 4 cm long from the oral cavity, and also sore throat, partial voice loss and vomiting. The polyclinic doctor suspected that the worm was a parasite but he could not make a diagnosis, and the patient was referred to Health Research and Training Hospital, Yüzüncü Yıl University. The parasite was examined in the Parasitology Laboratory and it was observed that this parasite was the nymph of *L. serrata* whose mouth was surrounded with four hooks and had approximately 90 body segments with small hooks (Yilmaz *et al*., 2011).

2.20.5 Iran

A 32-year-old woman suffering from upper respiratory symptoms was admitted to the respiratory tract clinic at the Afzalipour Hospital of Kerman University of Medical Science in the city of Kerman, the center of Kerman province, south- eastern Iran in 2013. She used to consume raw liver of sheep for many years, a traditional belief for its nutritional values. In microscopic examination of the nasopharyngeal discharge, *L. serrata* was detected (Yazdani *et al*., 2014).

Another specimen was isolated from a 10 year old resident in Shahrair County in Tehran province, with presentation of discomfort and prickling sensation in the throat, sneezing, coughing, and nasal discharge following the consumption of hamburger in the last day and undercooked liver (Anaraki *et al.*, 2008).

2.20.6 China

A 53 year-old male native of Shanghai died of heart failure because of cardiovascular syphilis. During the postmortem examination many protruding ‘pearls’ (<1 cm in diameter) were found incidentally over the serosal surface of the small intestine and the capsules of liver and spleen with extensive adhesions to the diaphragm. Histological examination revealed neither the embedded pentastomid nymph nor pathognomonic hallmarks of pentastomiasis or any of the pathological signs of tuberculosis infection. However, in the protruding ‘pearls’, characteristic concentric targetoid calci cations were found, each in the core of caseation with hyalinized capsule (Ma *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was done in Zaria metropolis. Zaria is a major city in Kaduna State in Northern Nigeria, as well as being a Local Government Area. Formerly known as Zazzau, it was one of the original seven Hausa city-states. The 2006 Census population was 975,228 and a density of 1,400/km^2. It is located within latitudes 11°04′N 7°42′E and longitudes 11.067°N 7.700°E with an altitude of 675 meters above sea level and a total land area of 300 km^2 (100 sq mi).

The old part of the city, known as Birnin Zaria or Zaria-City, was originally surrounded by walls, which now have been mostly removed. In the old city and the adjacent Tudun Wada neighbourhood people typically reside in traditional adobe compounds. These two neighborhoods are predominately occupied by the indigenous Hausa. The neighborhoods of Samaru and Sabon Gari are predominately occupied by Nigerians of southern origin, such as the Ibo. The largest marketplace is in Sabon Gari. Other more recent neighborhoods include: Danmagaji/Wusasa, PZ, Kongo, GRA-Zaria, Hanwa, Basawa, Lowcost Kofan-Gayan and Shika.

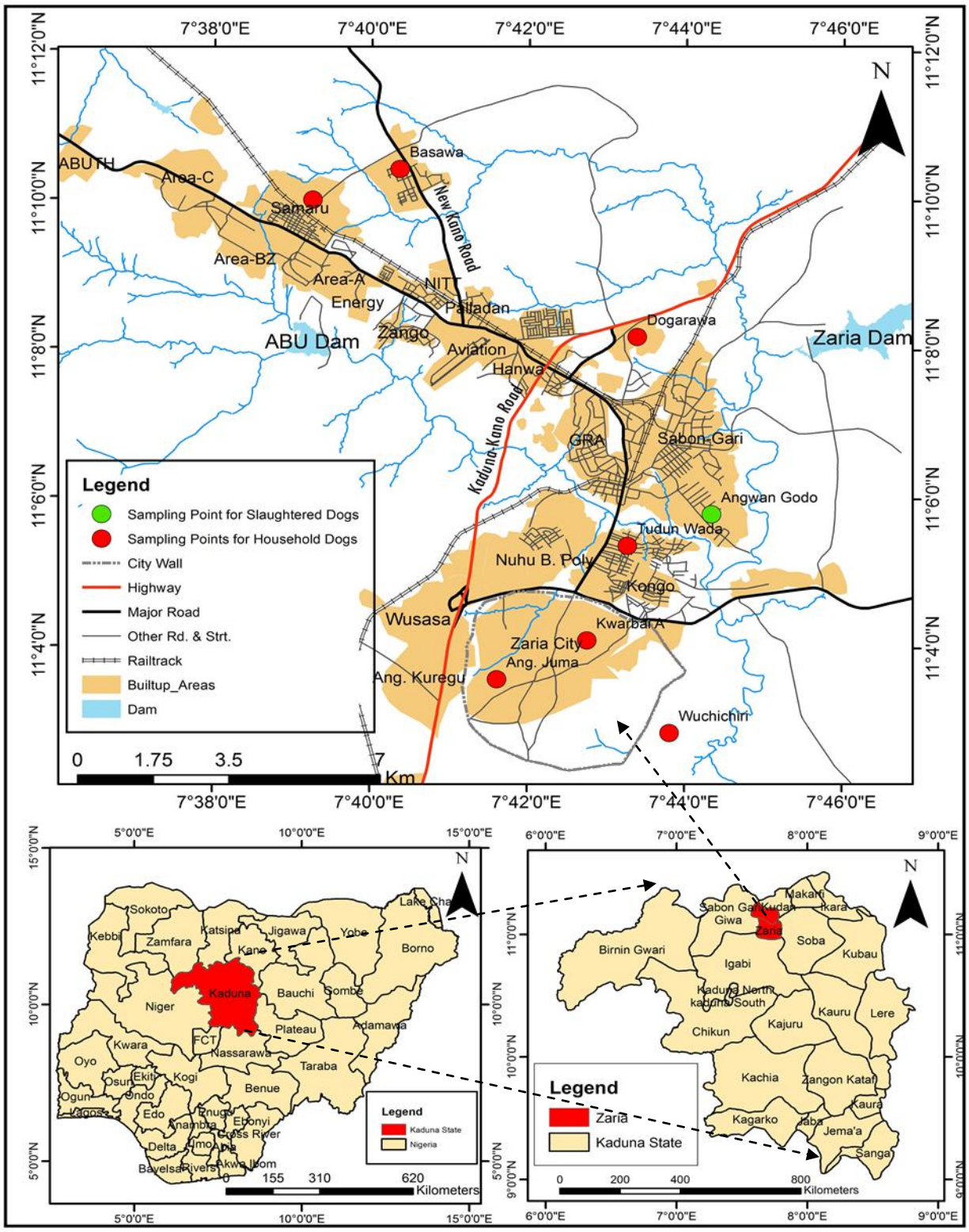


Figure 3.1: Zaria Urban Area showing the Sampling Points

Source: Quickbird, 2012.

3.2 Study Design

Cross sectional study was conducted in which household dogs and dogs slaughtered for human consumption were selected for this study. For prevalence of *L. serrata* adult worms in nasal passage, a dog slaughter location within the study area was selected as sampling unit. This location is Anguwan Godo in Sabon Gari area of Sabon Gari LG. And for the prevalence of *L. serrata* eggs in faeces and nasal secretions in dogs, 25% of wards in each local government, i.e Zaria and Sabon Gari LGAs were randomly selected, making 7 wards in all. These areas include Samaru, Dogarawa, and Basawa in Sabon Gari LGA and Kwarbai A, Wuchichiri, Anguwan Juma and Tudun Wada in Zaria LGA. An assistant who was conversant with the area was used in each ward to identify household that keep dogs, which were recruited for the study based on convenient sampling plan and consent of the owners.

3.3 Sample Size Determination

Sample size was determined by using the formular of Thrusfield (1997) at 95% confidence interval.

Using the prevalence of 37.45% by Oseni *et al.* (2014) sample size was calculated using the formular below;

$$N = \frac{Z^2pq}{d^2}$$

Where

N=sample size

Z= standard deviation at 95% confidence interval (1.96)

p= prevalence (37.45%), Oseni *et al*., 2014

= 0.3745

q=1-p

d= allowable error

$$N = \frac{1.96^2(0.3745 \times 0.6255)}{0.0025}$$

$$= \frac{3.84 \times 0.234}{0.0025}$$

$$= 360$$

From each ward, 57 dogs were sampled and in the seven wards a total of 399 dogs were sampled for this study. This was achieved by adding 10% of the calculated sample size to give 396 which was then rounded up to 399 for easy division and in order to obtain equal number of dogs sampled from each ward. A total of 399 faecal and nasal swab samples each were collected from household dogs and 360 heads were collected from slaughtered dogs.

3.4 Sample Collection and Procedure

Sampling was based on convenience sampling method where dogs sampled were selected based on availability and consent of owner. A scoop of faeces was collected from the rectum into sterile polythene bag and labeled. And also, the nostrils of the dogs were swabbed using a sterile swab stick and labelled. The samples were then placed in

Coleman's ice box and transported to the Helmintology laboratory of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria for analysis. For the detection of adult worm of *L. serrata*, based on availability of the slaughtered dogs, the heads were immediately separated from the carcasses, placed in Coleman's ice box and transferred to the laboratory.

3.4.1 Administration of Structured Questionnaires

Two hundred and thirty (230) structured questionnaires were administered to dog owners in these households (Appendix I) and all were retrieved. Information was generated on the respondent's demographic characteristics and risk factors that may predispose dogs and other herbivores to *Linguatula serrata* infection. The structured questionnaire was pre tested to determine its validity, corrections were made and applied before final copy was produced and used in the field.

3.5 Sample Processing

Zinc sulphate floatation technique as described by Cheesbrough, (1992) was used for the detection of the eggs (ova) of *L. serrata* in faeces. Zinc sulphate solution (specific gravity 1.18) was prepared in a glass beaker by adding 400 grams of granular zinc sulphate (crystals), 150 grams of sucrose, 1 gram thymol and 1000ml of distilled water. The water was heated to about 100^0C and poured into the mixture and stirred. The solution was then placed in a water bath and stirred continuously until the mixture had dissolved completely. The solution was then transferred to a 500ml cylinder and its specific gravity was adjusted to 1.18. The Zinc sulphate solution was transferred to a screw capped bottle, labeled, dated

and initialed and stored at 4^0C until used (Cheesbrough, 1992). Prepared solution was used within 7 days.

3.5.1 Examination of dog faeces for *L. serrata* eggs

About 1gram of the faecal sample was mixed with 5ml of distilled water in a beaker. It was then strained through a piece of gauze into a centrifuge tube and the tube was filled to about 2-3mm from the top with distilled water. This was then centrifuged at 5000rpm for 1 min and the supernatant discarded. Then, 1-2ml of zinc sulphate solution was added and the bottom of the tube was tapped sharply with a finger to resuspend the deposit. The tube was then filled to within 1-2mm from the top and centrifuge again at 5000rpm for 1 min. The centrifuge was allowed to come to a halt without disturbance. The tubes were removed and placed on a test tube rack and the floatation media was added to the filtrate until a positive meniscus was obtained. A cover slip was carefully placed on the preparation and allowed to stand for 3-5mins. The cover slip was carefully removed and placed on clean grease free glass slide and viewed under the microscope at x10 and x40 magnifications. Eggs of parasites seen were identified with the aid of an atlas, encyclopedia of parasitology and reference photographs (Jeffery and Leech, 1975; Bowman, 2000) and Manual of Parasitology (Jeffery and Leech, 1975; Cheesbrough, 1992).

3.5.2 Examination of nasal swab from dog for *L. serrata* eggs

Direct microscopy was carried out on nasal swabs. One to two drops of distilled water was placed at the center of a clean grease free slide. A smear of the nasal swab was made

directly on the slide and then covered with cover slip and viewed under the microscope for the presence of *L. serrata* eggs.

The confirmation of the egg (ova) was done through the study of its unique structure such as its ovoid shape, its brownish to yellowish color, and measure about 70x90 micrometers. The egg also contain a fully grow larva. Reference photographs (reference slide, atlas, googleimages.com) were also used to assist in confirmation.

3.5.3 Determination of infestation of slaughtered dogs with adult *L. serrata*

For the adult worm, the head of the dog was split longitudinally to expose the nasal and other cavities in the head. The nasopharynx area, nasal turbinate's, paranasal sinuses, Eustachian tubes, trachea, and brain were examined for the presence of adult form of *Linguatula serrata*. The tongue of the dogs were dissected sublingually, and the tissue samples consistent with the description of Oseni *et al*. (2014) were removed and subjected to gross and histopathological examination at the Histology laboratory, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The tissue was flattened, dehydrated in ascending grades of ethyl alcohol and cleared in creosote. It was then sectioned, mounted, stained by Haematoxylin and eosin staining technique and examined under light microscope at X200 and X400 magnifications.

3.6 Data Analysis

Statistical Package for Social Sciences (SPSS version 16.0) was used to analyse the data obtained and results were presented in form of tables and charts. Chi square, Fisher's exact tests, odds ratio and 95% confidence interval were used to show association between

variables (sex, age and breed) and infection and also for association between risk factors and infection with *L. serrata*. P values <0.05 was considered significant for these analyses.

CHAPTER FOUR

RESULTS

4.1 Prevalence of *Linguatula serrata* in Slaughtered Dogs in Zaria and Environ

4.1.1 Prevalence of *Linguatula serrata* in nasal cavity of slaughtered dogs

Of the 360 dogs examined for adult form of *Linguatula serrata* in slaughtered dogs, there was no parasite found in the nasal cavity and tongue.

4.1.2 Prevalence of *Linguatula serrata* in tongue of slaughtered dogs

Out of 360 dogs examined for the parasite in the tongue, 274 (76.1%) of the dogs had tissue material that resemble the description of *L. serrata* according to Oseni *et al.* (2014) in the ventrum of the tongue (Plate I). This tissue is a cordlike structure enclosed in a dense irregular connective tissue capsule and extends longitudinally, in the midline, near the ventral surface of the of the apex of the tongue. It runs from the center of the tip of the tongue for about 3-4cm beyond the attachment of the frenulum of the tongue. On histopathological examination, the tissue was found to be filled with skeletal muscle, white adipose tissue, dense irregular connective tissue capsule, intrinsic lingual muscles, blood vessels and nerves. The tissue material described as a tongue worm by Oseni *et al.* (2014) and local dog butchers is not consistent with the features of *Linguatula serrata*.

4.2 Prevalence of *Linguatula serrata* eggs in Faeces and Nasal Secretions of Household Dogs

4.2.1 Presence of *Linguatula serrata* eggs in faeces of household dogs

None of the 399 faecal samples examined from household dogs showed eggs of *Linguatula serrata* (Table 4.1).

4.2.2 Presence of *Linguatula serrata* eggs in nasal secretion of household dogs

Out of the 399 household dogs examined for *Linguatula serrata* eggs in nasal secretions, 10 were positive (Plate II) giving a prevalence of 2.5% (10/399), as shown in Table 4.1



Plate I: Head of a slaughtered dog split longitudinally showing nasal and other cavities of the head.

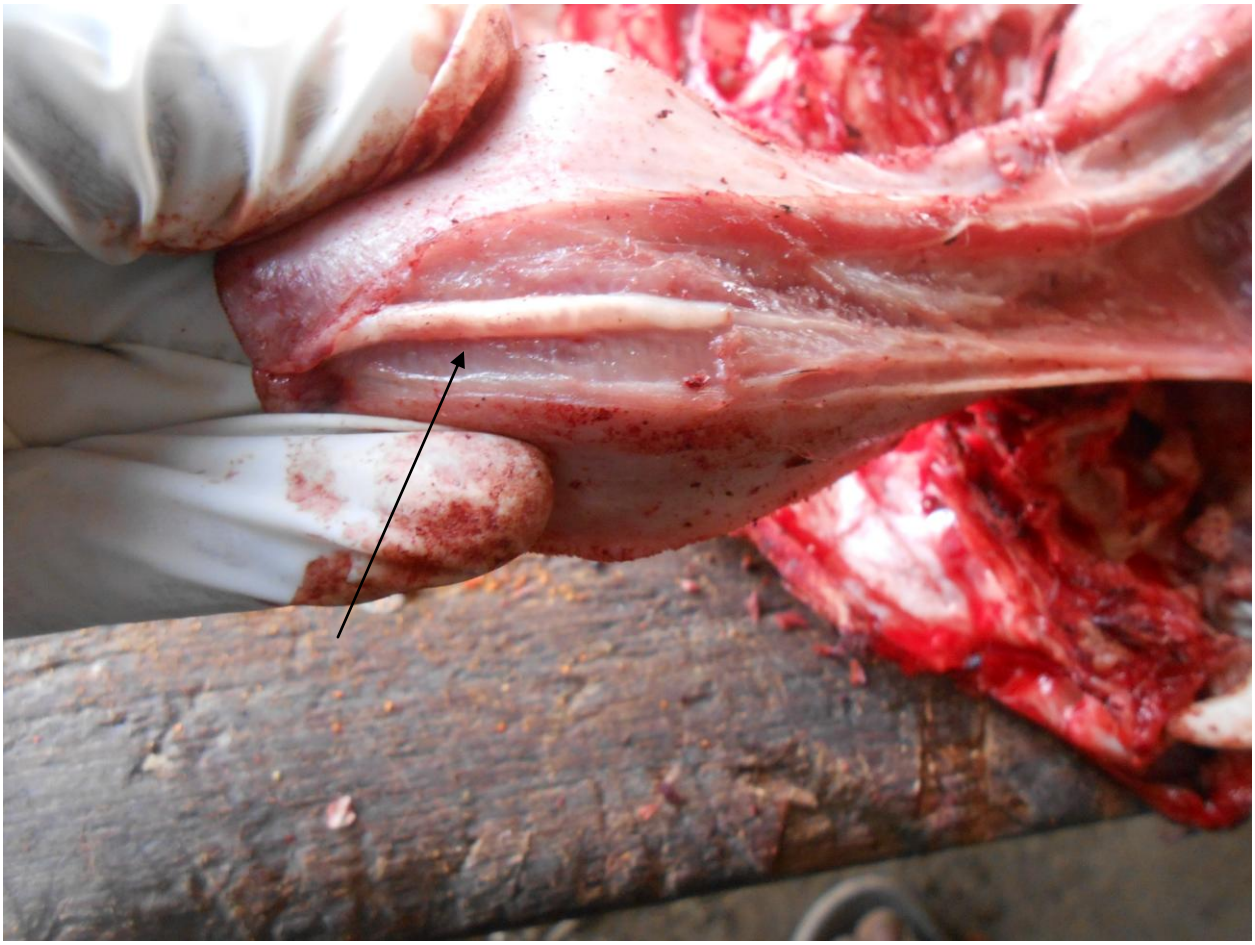


Plate II: Tongue of the dog dissected showing Lyssa fibrous connective tissue

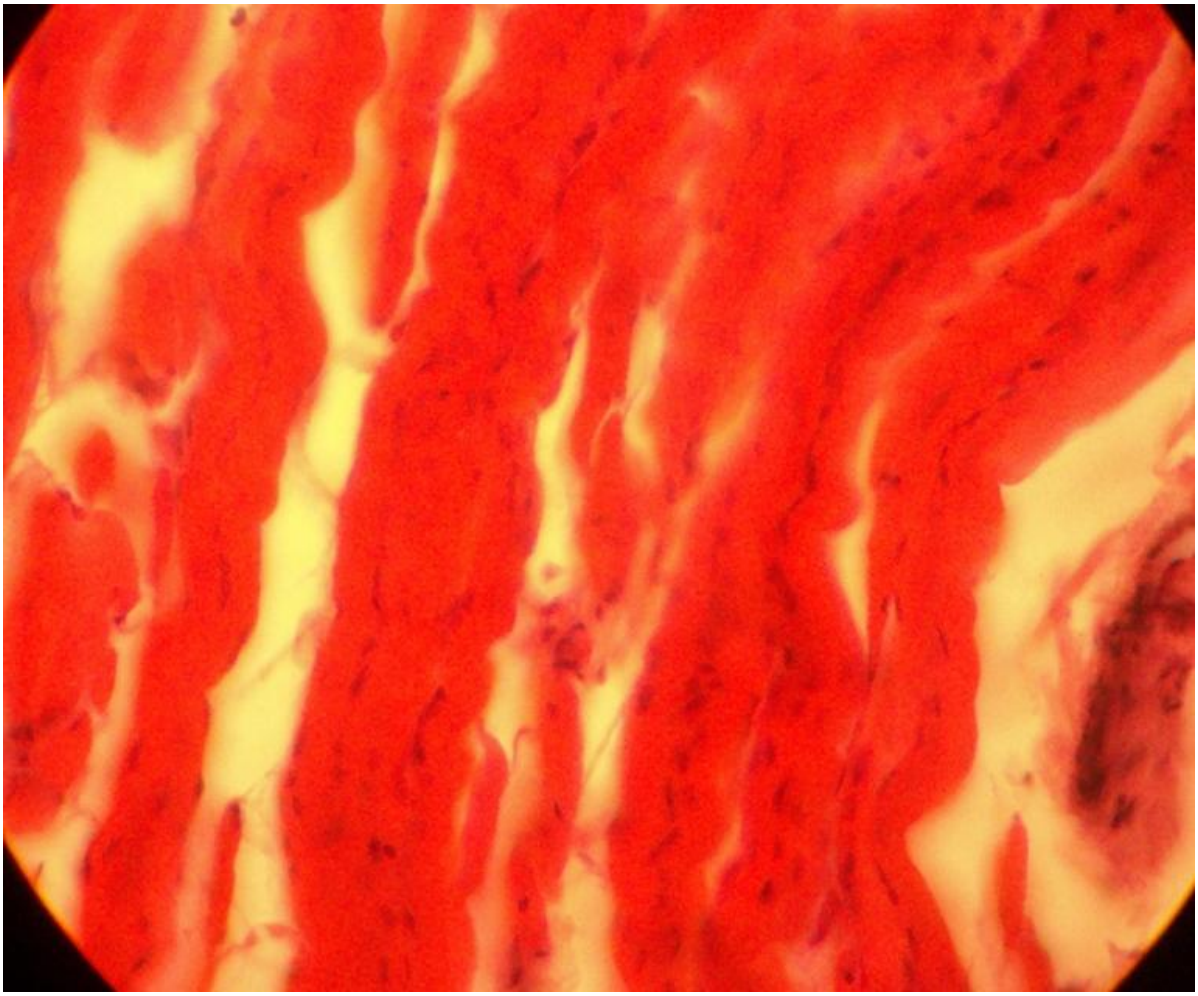


Plate III: Histopathological section of the tissue material (Lyssa) purported to be *L. serrata* in the ventrum of the tongue of the tongue (Haematoxylin and eosin, X400 magnification)



Plate IV: Matured egg of *L. serrata* recovered from the nasal secretion of dog (x400 magnification)

Table 4.1: Prevalence of *Linguatula serrata* in household dogs in Zaria and environ

Sample (dog)	No examined	No positive	Prevalence (%)
Faecal	399	0	0
Nasal	399	10	2.5

4.2.3 Prevalence of *Linguatula serrata* in nasal secretion based on sex of dogs

Based on the sex of dogs examined, there was an equal number of positive for both sexes, 5, with a prevalence of 2.1% and 3.0% for males and females respectively. There is no significant difference between the two sexes and infection with *Linguatula serrata* ($P > 0.05$). Table 4.2

4.2.4 Prevalence of *Linguatula serrata* in nasal secretion based on age of dogs

The prevalence of *Linguatula serrata* across different age groups is shown in Table 4.3.Out of the 399 dogs examined, 70 were under the age of 1 and only 1 dog was found to be infected with *Linguatula serrata* eggs with a prevalence of 1.4% while 6(3.5%) out of 172 dogs aged 1-2 years yielded the parasite. Also 3(1.9%) of the 157 dogs aged above 2 were positive for *Linguatula serrata*. There was no significant difference between the age groups and infection with *Linguatula serrata* ($P > 0.05$).

4.2.5 Prevalence of *Linguatula serrata* in nasal secretion based on the breed of dogs

Based on the breed of dogs, out of 399 dogs examined, all positive to *Linguatula serrata* were from the local breed, were 366 were examined, giving a prevalence of 10(2.5%). Out of the 21 and 12 examined for exotic and local breeds respectively, none was found to be infected with *Linguatula serrata*. Here also, there was no significant association between breed of dog and infection with *Linguatula serrata* ($P> 0.05$). Table 4.4

Table 4.2: Prevalence of *Linguatula serrata* in nasal secretion based on sex of dogs in Zaria and environ

Parameter	Variables	No Examined	No positive	Specific rate (%)	OR	95% CI	P value*
Sex	Male [ref]	233	5	2.1	1	0.201- 2.479	0.585
	Female	166	5	3.0	0.706		
Total		399	10	2.5			

*Chi square

Table 4.3: Prevalence of *Linguatula serrata* in nasal secretion across age groups of dogs

Parameter	Variables	No Examined	No positive	Specific rate (%)	P value*
	<1	70	1	1.4	
Age	1-2	172	6	3.5	0.633
	>2	157	3	1.9	
Total		399	10	2.5	

*Fisher's exact test

Table 4.4: Prevalence of *Linguatula serrata* in nasal secretion based on breed of dogs

Parameter	Variables	No examined	No positive	Specific rate (%)	P value*
Breed	Local	366	10	2.7	1.000
	Exotic	21	0	0.0	
	Cross	12	0	0.0	
Total		399	10	2.5	

*Fisher's exact test

4.2.6 Prevalence of *Linguatula serrata* in nasal secretion based on purpose of keeping dogs

When the occurrence of the parasite was recorded based on the purpose the dogs were kept, 95 dogs kept for security purpose and 22 kept as pets recorded no positive for *Linguatula serrata*. Those kept for the purpose of hunting had a prevalence of 3.0% (n= 7) while those who are kept for more than one purpose had a prevalence of 6.1% (n= 3). In this case however, there was no significant association between the purpose of keeping the dogs and infection with *Linguatula serrata* (P> 0.05) (Table 4.5)

Table 4.5: Prevalence of *Linguatula serrata* in nasal secretion based on the purpose for which dog's are kept

Parameter	Variables	No examined	No positive	Specific rate (%)	P value*
Purpose of Keeping dog	Security	95	0	0.0	0.107
	Hunting	233	7	3.0	
	Pet	22	0	0.0	
	More than 1	49	3	6.1	
Total		399	10	2.5	

*Fisher's exact test

4.3 Results of assessment of risk factors for *Linguatula serrata* infection in household dogs

4.3.1 Demographic features of the Respondents

Two hundred and thirty structured questionnaires were administered to the dog owners in the study areas. Up to 92.2% (212/230) of the respondents were males while 7.8% (18/230) were females. 43 (18.7%) of the respondents fall in the <20 years age category, 59.1% (136/230) are between 20-39 years, 20% (46/230) are between 40-59 years, while just 2.2% (5/230) are 60 years and above. Among these respondents, 55.2% (127/230) were married while 44.8% (103/230) were single. Based on their educational qualification, 12.6% (29/230), 52.6% (121/230), 16.1% (37/230) and 18.7% (43/230) had their primary, secondary, tertiary, and other form of education respectively (Table 4.7).

4.3.2 Other informations on dogs from respondents

Based on the number of dogs owned by each respondent 45.7% (105/230), 38.3% (88/230), 13.5% (31/230) and 2.6% (6/230) respondents have 1, 2, 3 and 4 dogs respectively. Majority of respondents do not patronize veterinary clinics or hospitals for their dogs, 56.1% (129/230), followed by those that patronize yearly 23.5% (54/230) and then quarterly 17.8% (41/230) and the lowest on monthly basis 2.6% (6/230). For the reason of visit to veterinary clinic or hospital, 7.4% (17/230), 13.0% (30/230), 0.9% (2/230) and 23.0% (53/230) take their dogs for deworming, vaccination, disease or injury and more than one reason respectively.

Seventy two (72) respondents observed frequent sneezing from their dogs while 68.7% (158/230) did not observe sneezing in their dogs. Very few respondents have ever notice an

unusual material or nasal secretion from the nostril of their dogs 3(1.3%) with about 98.7% (227/230) who did not observe any unusual material or nasal secretion from their dogs (Table 4.7).

4.3.3 Prevalence due to risk factors that may predispose to *Linguatula serrata* infection in dogs

Other risk factors associated with management include feeding of raw meat (OR 0.507, 95%CI 0.141-1.824, P= 0.343), with more risk for those that don't (3.4%) than those that do (1.8%), scavenging (OR 0.968, 95% CI 0.948-0.988, P= 0.126) with more risk for those that do (3.2%) than those that don't (0%), and restriction (OR 0.966 95% CI 0.948-0.988 P= 0.068) with more risk for those allowed to freely roam on the street (3.4%) than those kept indoors or caged (0%). There was no statistically significant association between these risk factors and infection to *L. serrata* (P>0.05) (Table 4.6).

When occurrence of the parasite was recorded based on dogs been raised with other animals, 6(4.8%) dogs out of 124 been raised with ruminants were positive for *Linguatula serrata* while 3(7.1%) out of 42 raised with poultry and only 1(2.0) of 49 raised with both ruminants and poultry were found to be positive. *Linguatula serrata* eggs were not recovered from dogs been raised with cats, poultry and cats, ruminants, poultry and cats and those raised alone. There was statistically significant association between dogs raised with other animals and *Linguatula serrata* infection (P = 0.03) (Table 4.6).

Table 4.6: Demographic characteristics of respondents (dog owners)

Variable		Number	Percentage (%)
Gender	Male	212	92.2
	Female	18	7.8
Age (years)	<20	43	18.7
	20-39	136	59.1
	40-59	46	20.0
	60 above	5	2.2
Marital status	Married	127	55.2
	Single	103	44.8
Educational Qualification	Primary	29	12.6
	Secondary	121	52.6
	Tertiary	37	16.1
	Others	43	18.7
No of dog(s) Owned	1	105	45.7
	2	88	38.3
	3	31	13.5
	4	6	2.6
Vet patronage	Monthly	6	2.6
	Quarterly	41	17.8
	Yearly	54	23.5
	Never	129	56.1
Reason for Visit	Deworming	17	7.4
	Vaccination	30	13.0
	Disease or injury	2	0.9
	More than 1	53	23.0
Frequent Sneezing	Yes	72	31.3
	No	158	68.7
Unusual material From nostril	Yes	3	1.3
	No	227	98.7

*Dog owners

Table 4.7: Occurrence of risk factors that may predispose to *Linguatula serrata* infection in dogs

Factors	No Examined	No positive	Specific rate (%)	OR	95% CI	P value*
Feeding of raw meat						
Yes[ref]	225	4	1.8	1		0.343
No	174	6	3.4	0.507	0.141-1.824	
Scavenging						
Yes[ref]	311	10	3.2	1		0.126
No	88	0	0.0	0.968	0.945-0.987	
Restriction						
Freely on street[ref]	292	10	3.4	1		0.068
Around compound/ caged	107	0	0.0	0.966	0.945-0.987	
Raised with Other animals						
Ruminants	124	6	4.8			
Poultry	42	3	7.1			
Cats	10	0	0.0			
Poultry and cats	7	0	0.0			
Ruminants and poultry	49	1	2.0			0.035
Ruminants, Poultry and cats	6	0	0.0			
Alone	161	0	0.0			

*Fisher's exact test

4.3.4 Prevalence of *Linguatula serrata* based on wards sampled

Out of the 57 dogs sampled from Dogarawa ward, 6 were found to be positive giving a prevalence of 10.5%. However, 3(5.3%) were positive in Tudun wada while only 1(1.8%) was positive in Basawa. The parasite was not found in dogs in Samaru, Wuchichiri, Anguwan juma and Kwarbai A wards. There is a significant association between the location and infection with *Linguatula serrata* with P value (P= 0.001) (Table 4.8).

4.4 Other parasites found in faeces of household dog(s)

Other parasite's egg(s) of public health importance were found in the faeces of dogs examined. These parasites are, *Ancylostoma* spp 20(5.0), *Ascaris* spp 1(0.3), *Ascupularis* spp (0.3), *Balantidium coli* 1(0.3), *Cystioisospora* spp 1(0.3), *D. caninum* 4(1.0), *Gucoleus* spp 1(0.3), *Isospora* spp 24(6.0), *Moneiza* spp 1(0.3), *Spirocerca* spp 17(4.2), *Taenia* spp 59(14.7), *Toxocara* spp 10(2.5) (Table 4.9).

Table 4.8: Prevalence of *Linguatula serrata* eggs in nasal secretion of dogs based on wards

Location (LGA)	Wards	No examined	No positive	Specific rate (%)	P value*
	Samaru	57	0	0.0	
Sabon gari	Basawa	57	1	1.8	
	Dogarawa	57	6	10.5	
	Wuchichiri	57	0	0.0	0.001
	Ang. juma	57	0	0.0	
Zaria	Kwarbai A	57	0	0.0	
	Tudun wada	57	3	5.3	
Total		399	10	2.5	

*Fisher's exact test

Table 4.9: Other parasites found in faeces of household dogs in Zaria and environ

Parasites	No of dogs Positive	Proportion (%)
Helminth		
Ancylostoma spp	20	5.0
Ascaris spp	1	0.3
Ascupularis spp	1	0.3
Taenia spp	59	14.7
Toxocara spp	10	2.5
Diphylidium caninum	4	1.0
Moneiza spp	1	0.3
Protozoan		
Balantidium coli	1	0.3
Cystioisospora spp	1	0.3
Gucoleus spp	1	0.3
Isospora spp	24	6.0
Spirocerca spp	17	4.3
No parasite found	259	64.9
Total	**399**	**100**

CHAPTER FIVE

DISCUSSION

Linguatula serrata is a cosmopolitan species and both larval and nymphal stages have been recorded from human in Africa, Europe and the Americans (Schmidt and Roberts 1989). There are limited studies on the occurrence of *L. serrata* in Nigeria. Recent studies established an endemic state of the parasite in different parts of Nigeria (Nwosu *et al*., 2002; Oseni *et al*., 2014). Although a recent study was carried out on the prevalence of the adult worm in nasal cavity of dogs by Oseni *et al*. (2014), in Jalingo, Taraba state, Nigeria, this study to the best of our knowledge, may be the first to determine the prevalence of its infective eggs in faeces and nasal secretion of dogs in Nigeria.

L. serrata was not found in the nasal cavity of slaughtered dogs examined. This finding is contrary to the report of Oryan *et al.* (2007) who reported 76.5% prevalence in stray dogs in Iran, Rezaei *et al*. (2011) in North West Iran who reported 27.83% prevalence in dogs. Meshgi and Asgarian (2003) reported 62.2% prevalence in stray dogs also in Iran and also the report of Oseni *et al.* (2014) who recorded a 38.5% prevalence in 2010 and 35.67% in 2011 in Nigeria. Perhaps the wide difference may be due to the geographical location of the study area and the fact that there is more of exportation of dogs from this area than there are imported because the slaughter and consumption of dogs in this area is very minimal compared to other states in the country.

This findings of this work could not verify or corroborate the report of Oseni *et al*. (2014) who reported detecting *Linguatula serrata* sublingually (ventrum of the tongue). The tissue material consistent with the descriptions of Oseni *et al.* (2014) was found to be a fibrous

connective tissue named “Lyssa” in previous literature. This is due to its cordlike structure enclosed in a dense irregular connective tissue capsule and extends longitudinally, in the midline, near the ventral surface of the apex of the tongue as described by Horst and Hans-Georg (2007). Also described by Malcolm (1962) lyssa of the tongue of dog is made up of skeletal muscle, white adipose tissue, dense irregular connective tissue capsule, intrinsic lingual muscles at the ventral surface of the tongue, as seen under the microscope at x28 magnification when stained with Haematoxylin and eosin staining technique. In all previous literatures, adult *L. serrata* has never been reported in the tongue of dogs (Meshgi and Asgarian, 2003; Oryan *et al*., 2008; Rezaei *et al*., 2011). Dog handlers and owners also refer to this connective tissue as tongue worm which they believe causes in appetence in dogs.

Linguatula serrata eggs were also not detected in faecal samples of dogs examined which may be due its low prevalence in faeces. Ehrenford and Newberne (1981) reported from a 10 years study that only 7 cases of *L. serrata* infection were diagnosed out of approximately 18,500 faecal specimens collected from dogs, giving a prevalence of 0.04%. This finding is corroborated by the report of Gabriela Perez (2014) who reported that eggs of *L. serrata* may not be found in the faeces of dogs during faecal examination but can be found in nasal secretion. This finding is probably the first finding in Nigeria as a whole and no more other literature backing to support this assertion requiring further investigation to support or refute this finding.

An overall prevalence of 2.5% was established from nasal secretion of dogs in the study area. The finding is in support of preponderance of the parasite in nasal secretion reported

by Ehrenford and Newberne (1981) in mid western United States who reported 0.4% prevalence of *Linguatula serrata* in nasal secretion. This study recorded a higher prevalence than previous study conducted. Siavashi *et al.* (2002) also reported that linguatulid eggs can be diagnosed by microscopically identifying them from nasal mucus.

A prevalence of 2.1% and 3.0% was recorded for males and females respectively, with prevalence been higher in females than males which may probably be due to the fact that helminth egg shedding may be higher in female particularly pregnant ones. During the period around the birth of a litter, the immunity of the female dog to infection is partially suppressed. Due to immune system suppression at the time of whelping, active infection may occur in pregnant dog, with substantial number of eggs been shed. There is no statistically significant association between presence of parasite and sex. The finding is in agreement with Saeid *et al.* (2010) who reported that sex is not important in *L. serrata* infection since animals of the same age group do not show difference across sex. This however does not agree with the isolated report of Razaei *et al*. (2012) who reported a lower infection rate in female than male.

Majority of dogs sampled were found to be between 1-2 years age group and were shown to have the highest prevalence of 3.5% compared to those in older age group (>2) with a prevalence of 1.9%. However, the prevalence rate was lower in dogs less than 1 year of age, 1.4%. There was no significant association in the rate of infection and different age groups of dogs. This agrees with the finding of Meshgari and Asgarian (2003) and Oryan *et al.* (2007) but differed with the findings of Razaei *et al.* (2012) in which significant differences was found between the rates of infection in dogs of different ages. It is

postulated that the low prevalence of linguatulosis in young puppies is associated with their long maturation period (> 6 months) (Tavassoli *et al.,* 2001) and also feeding on mother's milk which obviates the need to feed them with the infected offal of ruminants (Oryan *et al.,* 2008) which minimize their exposure to the parasite. The presence of high rate of infection in age range 1-2 years likely shows easy accessibility of these animals to the source of infection.

The infection rate was higher in the local breed with a prevalence of 2.5% compared to exotic and cross breeds which turned out to be negative. This could be attributed to the fact that exotic and mostly cross breeds are kept by rich and affluent people where the animals have better veterinary care and restriction to outdoor environment and contact with ruminants. There was no significant association between breed and infection with *Linguatula serrata.*

A high prevalence was recorded in dogs that are mostly kept for more than 1 purpose which is mostly hunting and security, 6.1%, followed by those kept for hunting purpose with a prevalence of 3.0%. This may be due to the fact that they are used by Fulani tribes as guard for their herds and also for hunting during migration and sometimes this dogs are fed uncooked offals from dead or slaughtered animals (Saberfiroozi *et al*., 1998). No record of infection was seen in dogs that are kept mainly as pet or just for security purpose in homes, which may also be attributed to dogs been kept caged or usually indoors which limits their proximity to domestic animals and scavenging.

The major risk factors that predispose to *Linguatula serrata* infection in dogs is mostly feeding of raw meat or visceral, scavenging and restriction of dogs. There was an

association between occurrence of *L. serrata* infection and feeding of raw meat (OR 0.507, 95% CI 0.141-1.824), scavenging (OR 0.968, 95% CI 0.954-0.987) and restriction of dogs (OR 0.966, 95% CI 0.945-0.987). There was no statistically significant association between these factors and infection with *L. serrata* in dogs. The major reason for this prevalence of linguatulosis in dogs is that offals of domestic ruminants is the principal source of infection of domestic dogs and the ingestion of raw visceral of sheep, goats, and other herbivores resulted in high incidence of pentastomosis in dogs. Also dogs that are allowed to roam freely on the street and scavenge have access to slaughterhouse offals or the carcasses of infected herbivores such as sheep, goats and cattle, which may facilitate the spread of the parasite (Oryan *et al*., 2008). Soulsby (1982) also reported that feeding dogs with infected viscera was described to increase the incidence of linguatulosis.

Meanwhile, a prevalence of 4.8%, 7.1% and 2.0% was recorded in dogs been raised with ruminants, poultry and ruminants and poultry together respectively, with a statistically significant association (P = 0.03) between dogs raised with other animals and infection with *L. serrata*, which was similar to Oryan *et al*. (2007). This prevalence may be due to the close proximity of dogs and domestic animals in most household livestock units that may favour the contamination of feed and water intended for livestock with eggs of *L. serrata*. Also from a study by Razaei *et al.* (2012), they reported about one third of dogs were found to be infected with *L. serrata* and the close contact between dogs and livestock explains the greater rate of infection in intermediate hosts (ruminants).

In other factors such as frequent sneezing and unusual material or nasal discharge from the nostrils, a prevalence of 2.6% was recorded for those who sneezing was observed which is

in agreement with Junquera (2014) who reported that in most cases infected dogs show no clinical signs only in massive infections. As for observation of unusual material or nasal discharge from the nostril, it was not observed in all dogs infected giving a prevalence of 2.5% which also agree with Junquera (2014) who reported that adult worms can be directly visualized in the nasopharynx through rhinoscopy or rhinotomy. Both of these factors show insignificant association between frequent sneezing and unusual material from the nostril of dog and infection with *Linguatula serrata* infection.

Based on location/wards been sampled for this study, the prevalence of *L. serrata* is higher in Sabon Gari LGA with 10.5% and 1.8% for Dogarawa and Basawa respectively and only 5.3% for Tudun Wada ward in Zaria LGA. This may be attributed to easy access of dogs to offals and carcasses of ruminants from slaughterhouses located in these areas and these dogs are also allowed to roam freely on the street and scavenge. The data from this study cannot sufficiently explain these findings. Further work may be required to fully establish the reason for the differences in these areas.

Majority of the respondents (92.2%) were male, this is associated with the sampling area since male are the majority owners of household dogs where most of the questionnaires were administered. Out of the respondents of the questionnaires (59.1%) were between the ages of 20-39, this may be because majority of the population falls within this age group.

And among these respondents, 55.2% are married which may be because only those that are married are likely to be household owners. So also, 52.6% of respondents answered to only have secondary school education as their highest level of education with a significant

proportion having higher education. These levels of education are sufficient to understand public health messages and risks in disease control.

Majority of respondents owned only 1 dog (45.7%) and most of them do not patronize veterinary clinic or hospital (56.1%) often dog owners only seek for veterinary care when the dog is very ill rather than for prevention. Only (23.0%) take their dogs for routine vaccination and deworming. This group consisted of dog owners who keep their dogs for security purpose or as pet. This is because most of these dogs are either exotic or crosses and are usually owned by educated and rich people (Oseni *et al*., 2014).

Most of the respondents (68.7%) did not observe frequent sneezing in their dogs and also 98.7% have not ever observed any unusual material or nasal secretion from their dogs, linguatulosis is one of the causes of frequent sneezing among other endemic diseases of dogs. Often linguatulosis can be asymptomatic and go undetected (Siavashi *et al*., 2002).

With the prevalence reported in this study, the public health implication in a country like Nigeria where cattle, goat and sheep meat are quite popular among the local population, the consequences will be far reaching.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Adult *L. serrata* was not detected in the nasal cavity of slaughtered dogs examined in Zaria. *L. serrata* eggs were also not detected in the faeces of dogs examined but were found in the nasal secretion of dogs with an overall prevalence of 2.5%. There was no statistically significant association between sex, age, breed and purpose for which dogs are kept and infection with *L. serrata*. (P> 0.05). There was statistically significant association between *L. serrata* infection with sample location and raising of dog with other ruminants. There was also no statistically significant association between occurrence of *L. serrata* and risk factors such as feeding of raw meat, scavenging and restriction of dogs (P >0.05). This study being the first to be done in Zaria, Kaduna state, Nigeria has established the presence of this parasite in dogs, suggesting the possibility of infection in man and other animals in this environment.

6.2 Recommendations

1. Security measures should be improved upon in the abattoir by the authorities in order to stop entrance of stray dogs into abattoir premises thereby limiting their access to carcasses of infected ruminants.
2. Veterinarians, dog handlers and owners are better advised to wash hands after handling or treating dogs regularly.
3. Dog owners should be advised not to feed their dogs with raw viscera of goats, sheep, or other herbivores.
4. Further research should utilize more sensitive diagnostic faecal examination techniques for possible detection of the eggs in faeces.
5. Since livestock are a major source of infection for humans and other carnivores, veterinarians should pay additional attention during meat inspection in order to enhance the detection of *L. serrata* infection in slaughterhouses
6. Further studies should be conducted in both domestic and wild carnivores and herbivores in different parts of the country.
7. Medical authorities should be educated on the epidemiology of the parasite which may help to reduce the risk of infection.

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APPENDICES

Appendix I

**DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY ZARIA**

RESEARCH QUESTIONNAIRE ON: Risk factors that may predispose to *Linguatula serrata* infestation in dogs. This information you are providing will be treated as confidential and used only for academic activity. Please kindly provide the information to the best of your knowledge.

1. Date……. Location……..

OWNER'S DEMOGRAPHIC DATA

2. Gender
   Male [ ]
   Female [ ]

3. Age (years)
   <20 [ ]
   20-39 [ ]
   40-59 [ ]
   60 and above [ ]

4. Marital status
   Single [ ]
   Married [ ]
   Divorced [ ]
   Widowed [ ]

5. Level of education
   Primary [ ]
   Secondary [ ]
   Tertiary [ ]
   Others [ ]

INFORMATION ON OWNED DOG

6. Number of dogs owned……..

7. Sex
   Male No [ ]
   Female No [ ]

8. Age
   <1 [ ]
   1-2 [ ]
   >2 [ ]

9. Breed
   Local [ ]
   Exotic [ ]
   Cross breed [ ]

10. What purpose do you keep your dog for?
    Security [ ]
    Pet [ ]
    Hunting [ ]
    For more than 1 of the above [ ]

11. How do you raise your dog?
    Alone [ ]
    Raised with ruminants [ ]
    Raised with poultry [ ]
    Raised with cats [ ]

12. How often do you feed your dog with raw meat or offals?
    Often [ ]
    Frequently [ ]
    Once in a while [ ]
    Never [ ]

13. Do you allow your dog to scavenge?
    Yes [ ] No [ ]

14. Does your dog……
    Stays indoors (caged) [ ]
    Roam around the compound [ ]
    Goes out freely on the streets [ ]

15. How often do you take your dog to vet clinic/ visit by a vet doctor?
    Never do [ ]
    Monthly [ ]
    Quarterly [ ]
    Yearly [ ]

16. For what purpose?
    Routine vaccination [ ]
    Routine deworming [ ]
    For treatment of disease or injury [ ]
    For more than 1 of the above [ ]

17. Have you ever notice frequent sneezing from your dog?
    Yes [ ] No [ ]

18. Have you ever notice something unusual coming out from your dog's nostrils?
    Yes [ ] No [ ]

Appendix II

Reference photographs for Confirmation of the adult *L. serrata* and egg



Fig 4.1: Adult *Linguatula serrata* in nasal cavity of dog
Source: www.patologia-veterinaria.com

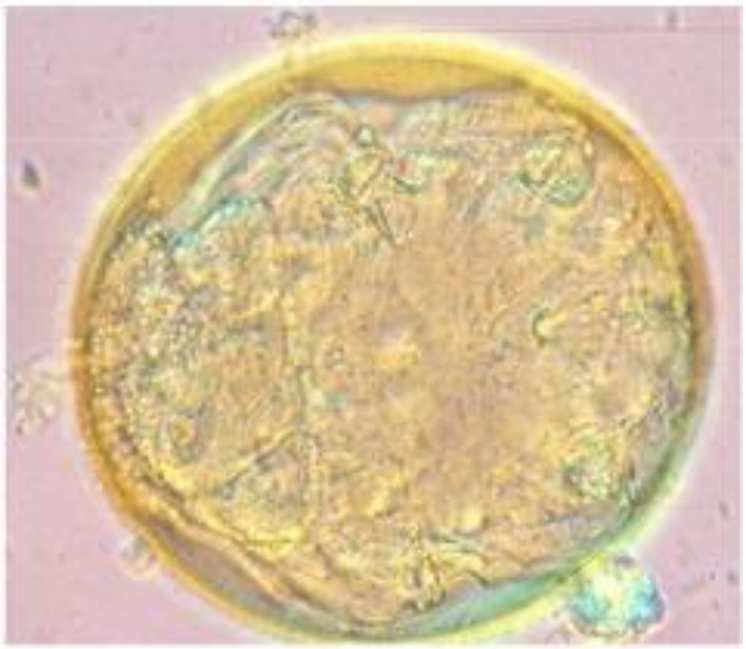


Fig 4.2: Reference photograph of matured *L. serrata* egg
Source: Googleimages.com