OCCURRENCE OF *CRYPTOSPORIDIUM* OOCYSTS IN CATFISH (*CLARIAS GARIEPINUS*) AND ITS HABITATS IN ZARIA, KADUNA STATE, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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NOVEMBER, 2017

DECLARATION

I declare that the work presented in this dissertation titled **"Occurrence of C*ryptosporidium* Oocysts in Catfish (*Clarias Gariepinus*) and its Habitat in Zaria, Kaduna State, Nigeria"** has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine. The information derived from literature has been 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.

Tawakalitu LAWAL **……** **…….**

Signature **Date**

CERTIFICATION

This dissertation titled **"Occurrence of *Cryptosporidium* Oocysts In Catfish (*Clarias Gariepinus*) And Its Habitat In Zaria, Kaduna State, Nigeria"** by Tawakalitu LAWAL meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its scientific contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to the Almighty Allah whose ultimate mercies, guidance and protection has brought me this far. It is also dedicated to my parents, late Alhaji Abdul Raheem and Maymuna Lawal as well as Alhaji and Hajia M.B. Lawal for giving me the basic training and to my husband Imran for his endless love and support.

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During this research work, I came across a number of people who in one way or the other contributed to its success, thus; I would like to use this medium to express my gratitude to my supervisors Dr. B. V. Maikai and Prof. M. Bello, for patiently guiding this work to its successful completion. Special thanks also to the laboratory technicians of the department of Veterinary Public Health and Preventive Medicine especially Mrs. Atawodi, Mr. Odoba, Mallam Yahuza and Mr. Martin as well as those of the helminthology laboratory of the department of Veterinary Parasitology and Entomology, especially Mr. Amadi and Mallam Yusuf.

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ABSTRACT

Cryptosporidium is an apicomplexan parasite known to cause gastrointestinal disease in humans and several animal hosts including piscines. To determine the occurrence of *Cryptosporidium* in catfish, *Clarias gariepinus*, and their aquatic habitats from the wild and cultured environments, 400 catfish from two dams (n = 100) and six backyard catfish farms (n = 300) as well as 40 water samples of 20L each from the dams (n = 10) and 10L each (n= 30) from six backyard farms were examined in Zaria, Kaduna State, Nigeria. The fish gut contents were concentrated using formol-ether concentration technique, whilst the water samples were concentrated using sedimentation and centrifugation techniques. All processed samples were stained with modified Ziehl-Neelsen method and morphometric measurements of oocysts taken with the aid of a calibrated eyepiece graticule. One hundred (100) questionnaires were also administered to catfish consumers within the study area, this was to obtain information on their level of awareness on diseases associated with catfish and their hygiene practices after handling catfish. Basic objectives of this study were to determine the followings; the prevalence of *Cryptosporidium* in catfish and its water habitat, the effect of the physico-chemical parameters of the water habitat of catfish on occurrence of *Cryptosporidium* in water sampled within study area, as well as the association between the prevalence of *Cryptosporidium* in *Clarias gariepinus* and management practices of catfish farmers in the study area. *Cryptosporidium* oocysts were found in 86 (21.5 %) out of the 400 fish, and 3 (7.5 %) out of the 40 water samples with oocysts of 3 different size ranges, corresponding to the following species; *C. molnari* – 50.0 % (4.69 µm ± 0.07 × 4.46µm ± 0.29), *C. parvum* – 45.3 % (5.05µm ± 0.12 × 4.49 µm ± 0.04), and *C. andersoni* – 4.7 % (7.40µm ± 0.47 × 5.60 µm ± 0.49). Of the four physico-

chemical parameters (temperature, pH, dissolved oxygen, and turbidity) measured, only turbidity was found to have a significant effect ($P < 0.05$) on the occurrence of *Cryptosporidium* in water sampled within the study area. Wild catfish were significantly more infected with *Cryptosporidium* oocysts than the cultured ones (OR = 1.962; 95% CI $1.204 < OR < 3.196$; $P = 0.0078$). *Cryptosporidium* oocysts were higher (10.0%) in dams as compared to 6.7% in water from backyard farms. The prevalence of *Cryptosporidium* oocysts in the bigger catfish within 451 to 850g was 28.0% compared to 21.1% in the smaller ones that were within 150 to 450g. Similarly, the prevalence was slightly higher (22.2%) in the lengthier (36 – 55cm) catfish than in the smaller ones (21.1%) within 15 – 35cm in length. The prevalence was also higher in male catfish (24.4%) than in females (19.2%). Based on management practices of catfish farmers, frequency of pond water replacement ($P = 0.0008$) and method of dead fish disposal ($P = 0.04$) were significantly more commonly associated with the occurrence of *Cryptosporidium* in backyard farms, whereas, type of pond, source of water, frequency of cleaning ponds and prophylactic medication to fish were not significant ($P > 0.05$). A significant number of the catfish consumers were unaware of any disease associated with catfish consumption (70.0%) or the zoonotic risks of any disease of catfish (86.0%). The presence of *Cryptosporidium* oocysts in catfish and their water habitats in the study area is of public health concern indicating the possible health threats posed to consumers and handlers.

TABLE OF CONTENTS

TITLE PAGE i

DECLARATION ii

CERTIFICATION iii

DEDICATION iv

ACKNOWLEDGEMENTS v

ABSTRACT vi

TABLE OF CONTENTS viii

LIST OF FIGURES xii

LIST OF TABLES xiii

LIST OF APPENDICES xv

LIST OF ABBREVIATIONS xvi

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Background of the Study 1

1.2 Statement of the Research Problem 3

1.3 Justification of the Study 5

1.4 Aim and Objectives of the Study 7

1.4.1 Aim of the Study 7

1.4.2 Objectives of the study 7

1.5 Research Questions 7

CHAPTER TWO 9

2.0 LITERATURE REVIEW 9

2.1. Introduction 9

2.2 Taxonomy of *Cryptosporidium* 11

2.3 Molecular Epidemiology of Cryptosporidiosis 14

2.4 Epidemiology of Cryptosporidiosis in Humans and Animals 16

2.5 Cryptosporidiosis in Animals 18

2.6 Waterborne Cryptosporidiosis 22

2.6.1 Contributing factors for the spread of water borne cryptosporidiosis 24

2.6.2. Monitoring and detection *Cryptosporidium* oocysts in water 26

2.7. Transmission of cryptosporidiosis 27

2.8 Morphology and life cycle *Cryptosporidium* 28

2.9 Laboratory diagnosis of cryptosporidiosis 30

2.9.1 Concentration of oocysts 31

2.9.2. Conventional staining methods for detection of *Cryptosporidium* oocysts 33

2.9.3 Measurement of oocysts 35

2.10 Prevention and Control of Cryptosporidiosis 36

2.12 Parasites of the African Catfish in Nigeria 40

CHAPTER THREE 43

3.0 MATERIALS AND METHODS 43

3.1 Study Area 43

3.2 Study Design 45

3.3 Sample Size Determination 46

3.4 Use of Questionnaire 47

3.5.1 Fish sample collection 47

3.5.2 Water sample collection 48

3.6 Preparation of Fish Samples 48

3.6.1 Euthanasia of fish 48

3.6.2 Determination of fish parameters 48

3.7 Fish Intestinal Sample Collection 49

3.8 Laboratory Procedures 49

3.8.1 Determination of the physco-chemical parameters of water 49

3.8.2 Concentration of water samples 50

3.8.3 Concentration of intestinal samples 51

3.8.4 Staining using modified Ziehl–Neelsen method 51

3.8.5 Calibration of oocyst size 52

3.9 Data analysis 53

CHAPTER FOUR..... 54

4.0 RESULTS 54

CHAPTER FIVE 76

5.0 DISCUSSION 76

CHAPTER SIX 90

6.0 CONCLUSION AND RECOMMENDATION 90

6.1 Conclusion 90

6.2 Recommendations 91

APPENDIX I 111

APPENDIX II 114

LIST OF FIGURES

Figure 3.1: Zaria Urban Area showing sampling locations of the study. 44

Figure 3.2: Schematic presentation of the study design 45

Figure 4.1 Level of awareness of consumers on catfish harboring infectious organisms ... 75

LIST OF TABLES

Table 4.1: Prevalence of *Cryptosporidium* oocysts in catfish from backyard farms and dams within Zaria, Kaduna State, Nigeria 55

Table 4.2: Prevalence of *Cryptosporidium* oocysts in water from backyard catfish farms and dams within Zaria, Kaduna State, Nigeria 56

Table 4.3: Association between the prevalence of *Cryptosporidium* oocysts in catfish and their water in Zaria, Kaduna State, Nigeria..... 57

Table 4.4: Association between the prevalence of *Cryptosporidium* oocysts and sources of catfish in Zaria, Kaduna State, Nigeria..... 59

Table 4.5 Mean of physio-chemical parameters of water samples examined in Zaria, Kaduna State, Nigeria 60

Table 4.6 Effect of the physico-chemical parameters of water on the occurrence of *Cryptosporidium* in water from the sampled units in Zaria, Kaduna State, Nigeria..... 61

Table 4.7: Morphometric measurements of 106 oocysts observed from 89 samples, in comparison with standard ranges and means of oocysts of corresponding *Cryptosporidium* species..... 64

Table 4.8: Distribution of oocysts and corresponding *Cryptosporidium* species in catfish and their water from backyard farms and in Zaria, Kaduna State, Nigeria..... 65

Table 4.9: Association between the prevalence of *Cryptosporidium* infection and length, weight and sex of catfish in Zaria, Kaduna State, Nigeria..... 69

Table 4.10: Association between the prevalence of *Cryptosporidium* infection and management practices in backyard catfish farms in Zaria Kaduna State, Nigeria..... 70

Table 4.11 Demographic information of catfish consumers in Zaria, Kaduna State, Nigeria..... 73

LIST OF APPENDICES

Appendix I: Questionnaire ……..108

Appendix II: Plate…….110

LIST OF ABBREVIATIONS

g/m^3 → gram per cubic metre

FITC → Fluoriscein Isothiocyanate

UV → Ultra violent

nm → Nanometre

mm → Millimetre

SI →System International

Xg → Revolution

SPSS → Statistical Package for Social Sciences

o_C → Degree Celsius

Mg/L → Milligram/litre

RNA → Ribonucleic acid

SSU → Small sub unit

Bp → Base pairs

Sspl → Streptomyces species

Vspl → Vibrio species

SAF → Sodium acetate – acetic acid formalin

PVA →Polyvinyl alcohol

ELISA →Immunological based assays

EAI →Enzyme Immunoassays

FAST → Falcon assay

IFAT → Immunofluorescence antibody test

CF → Complete fixation

Cm → Centimetre

n → Sample size

L → Litre

μm → micrometer

μl → microlitre

C I → Confidence Interval

g → gram

OR → Odds ratio

NTU → Nepholometre Turbidity Unit

PCR – RFLP → Polymerase Chain Reaction Restriction Fragment Length Polymorphism

DNA → Deoxyribonucleic acid

MZN → Modified Ziehl – Nelseen

AP → Aursamine phenol

HIV/AIDs → Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome

SD → Standard deviation

Spp → Specie

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Cryptosporidium is a protozoan parasite that is widespread among vertebrates causing mainly gastrointestinal disease in mammals and reptiles and enteric, renal and respiratory disease in birds (Chalmers and Giles, 2010). Although, infections have been reported in fish and amphibians, the disease has not been well described (Chalmers and Giles, 2010). Due to the wide host range of this parasite, cryptosporidiosis has been considered to be a zoonotic disease for some time now (Xiao and Feng, 2008). Species definition and identification of this genus is constantly changing, with the addition of "new" species based primarily on molecular criteria (Xiao and Feng, 2008). Currently, 32 species of *Cryptosporidium* are considered as valid species (OIE, 2016). *Cryptosporidium* spp recognized as piscine species include; *Cryptosporidium molnari, C. huwi and C. cichlidis* (Rona *et al.*, 2011; Certad *et al*., 2015; OIE, 2016).

The first case of human cryptosporidiosis was first described in 1976 in a three and a half year old girl who developed self-limited enterocolitis (Nime *et al.,* 1976). By the early 1980s, outbreaks among veterinary students and workers demonstrated the risk to animal handlers (Current *et al.,* 1983; Jakipii *et al.,* 1983) and later a massive water-borne outbreak in Milwaukee, Wisconsin in 1993 was reported by Mackenzie *et al.* (1995a). In developing countries, *Cryptosporidium* infections occur mostly in children younger than five years, with peak occurrence of the infections and diarrhoea in children younger than two years (Bhattacharya *et al.*, 1997; Bern *et al.*, 2000). This is particularly because of the

independent and synergistic effects of immune naivete, malnutrition and HIV infection (Siobban and Saul, 2008). Children can have multiple episodes of cryptosporidiosis, indicating that acquired immunity to *Cryptosporiidum* infection is short lived or incomplete. Early childhood cryptosporidiosis has been associated with subsequent impairment in growth, physical fitness and cognitive function (Siobban and Saul, 2008).

The organism is highly infectious with a medium infective dose (from human trials) of only 132 oocysts, and 20% of human subjects could be infected with as few as 30 oocysts. The oocysts are highly resistant to inactivation in the environment and there is however, no chemical method of inactivation to kill the organism in drinking water (Quiroz *et al*., 2000).

Transmission is faeco-oral, either through direct contact with infected hosts or through multiple vehicles including changing diapers, drinking water and food (Casemore, 1990). Understanding the hosts, sources of infection and transmission routes is vital for the control of this parasite for which specific treatment options are limited and vaccines lacking (Chalmers and Giles, 2010). Diagnosis is normally established by conventional microscopic methods and the modified Ziehl Nelseen (mZN) or auramine phenol (AP) methods using un-concentrated faecal smears (Casemore *et al.*, 1985; Casemore, 1991). The species of *Cryptosporidium* responsible for infection can be determined by PCR-RFLP or sequencing of *Cryptosporidium* DNA isolated from oocysts (Coupe *et al*., 2005).

Fish is one of the important sources of protein for humans and other animals in the tropics. According to FAO (1989), fish accounts for more than 40% of the protein diet of two-thirds of the global population. Fish does not only provide food for immediate

consumption, but people rely on fishing for economic gains and job provision. A well processed fish product from the tropics has a ready market in developed countries and is therefore a good foreign exchange earner (Imam and Dewu, 2010). Like other animals, fish is also afflicted by endo- and ectoparasites, especially protozoans and helminths causing heavy morbidity and mortality (Al-Murjan and Abdullahi, 2008). *Clarias gariepinus* commonly known as African catfish has been widely introduced around the world. These species are found as far as South Africa and Northern African regions. They have also been introduced in Europe, the Middle East, and parts of Asia. They are potamodromous, which means they migrate within streams and rivers. They are elongate with fairly long dorsal and anal fins. The dorsal fin has 61-80 soft rays and the anal fin has 45-65 soft rays. African catfish are subjected to a wide variety of diseases including bacteria, fungi and miscellaneous parasites (Madu et al., 1999).

1.2 Statement of the Research Problem

Cryptosporidium is one of the causes of diarrhoeal illness in man and animals worldwide (Xiao *et al.,* 2001a; Xiao and Feng, 2008; Maikai, 2009), and several groups of humans are particularly susceptible to it. In industrialized countires, epidemic cryptosporidiosis can occur in adults by food borne or water borne routes (Millard *et al.,* 1994; Mackenzie *et al.,* 1995b; Quiroz *et al.,* 2000). In immunocompromised persons, the incidence and severity of cryptosporidiosis increases as the $CD4^+$ lymphocyte cell count falls, especially when it falls below 200 cells/µl (Quiroz *et al.,* 2000). In severely immunocompromised patients, disease can progress to cholangitis or pancreatitis and the infection is frequently chronic and can eventually become lethal, second only to tuberculosis, thus, cryptosporidiosis is the main terminal disease in HIV infection (O'connor *et al.,* 2011). Two genotypes are the most

common causes of human cryptosporidiosis, and these are the zoonotic *C. pestis* (or the *C. parvum* "bovine genotype") and the human-specific *C. hominis* (or the *C. parvum* "human genotype") (Fayer, 2010).

Parasitic diseases of fish are of particular importance in the tropics. Parasites usually exist in equilibrium with their host as a survival strategy. However, in instances where hosts are overcrowded such as in aquaria or in fish farms, parasitic diseases can spread very rapidly causing high mortality. Fishes have been reported to be affected by some zoonotic organisms such *Mycobacterium*, in addition to which several protozoan parasites such as *Ichthyophthirius multifilii* have also been reported. Currently, three *Cryptosporidium* species are recognized in fish hosts namely: *C. molnari, C. scophthalmi* and *C. huwi* (Rona *et al*., 2011, Yang *et al*., 2016, Costa *et al*., 2016); other species such as the zoonotic *Cryptosporidium parvum* as well as *C. xiaoi* and *C. hominis* have also been reported in fish hosts (Koinari *et al.,* 2013; Ryan and Xiao, 2014; Certad *et al.,* 2015). Nonetheless, not much is known about *Cryptosporidium* species infecting piscine hosts, although it is known to cause high morbidity and a veriety of clinical signs (Alvarez-pellitero *et al.,* 2004; Ryan and Xiao, 2014; Ryan *et al.,* 2015; Yang *et al.,* 2015).

The African catfish *Clarias gariepinus* is a major tropical aquaculture species in Africa and the most popular with fish farmers and consumers in Nigeria. The total current catfish consumption in Nigeria according to the Farmers Association of Nigeria is about 1.5 million metric tons annually. Fish consumption is however, not devoid of risks due to the possibility of fish harbouring infectious or pathogenic microorganisms, particularly if such organisms are zoonotic (Leal *et al.,* 2008). Fish is an aquatic organism that can be processed into food for humans and animals; hence there are possibilities of parasites

harboured by fish to be transmitted to consumers and processors following improper cooking and careless handling, respectively.

Cryptosporidiosis is an emerging zoonotic gastroenteric disease that is known to occur in many animal species (Ayanda, 2009), including wild and farmed fish worldwide. Several reports have confirmed the cosmopolitan nature of *Cryptosporidium* species in surface water, groundwater, estuaries, and seawater. Its transmission is through its hardy oocysts which are passed in the faeces with water as its major vehicle of transmission. Thus, zoonotic transmission of cryptosporidiosis can occur from consumption of fish obtained from contaminated water (Leal *et al.,* 2008). In Nigeria, Maikai (2012), Atawodi and Bichi (2013) have reported the parasite in catfish. However, there is still need to investigate these findings further in relation to the fish habitat (water) and management practices of farmers as well as the level of awareness of the parasite among consumers in Zaria.

1.3 Justification of the Study

Fish is becoming an important component of people's diet in many parts of the world. This is probably due to its palatability, leanness, and possession of healthy Omega 3 fatty acids (Atawodi and Bichi, 2013). Realizing the importance of fish to human nutrition and its contribution to the national income makes it important to establish and strengthen aquaculture and fisheries programmes (Chilima, 2007), which is one of the reasons for the popularity of fish farming in Nigeria and around the world (Bernard, 2009). The importance attached to the culture of catfish in Nigeria is not only because it is a highly esteemed species that command high market value, but it is hardy and survives where most other cultivable species cannot (Maikai, 2012).

Zaria is located in the North-West part of Nigeria where extreme weather conditions can be experienced. This could be a reason for catfish being a major cultivated fish species in the area. The presence of fish ponds and several catfish retail outlets in Zaria is also an indication that the town is not left out in the global increase in catfish culture and consumption. Furthermore, some families engage in backyard catfish farming as a means of income supplementation. Thus, there is need to embark on this study to determine the various management practices in backyard catfish farms that may be associated with the occurrence of *Cryptosporidium* in cultured catfish and their habitats in Zaria.

Cryptosporidium has been reported as a prime candidate in transmission of water borne illnesses. It has also been found to be ubiquitous in surface waters and extremely resistant to various environmental pressures and chemical disinfectants (Perz *et al.,* 1998). Since *Cryptosporidium* has been reported in some animal species and vegetables within the study area, it is therefore, important to check the aquatic habitat in order to determine the possibility of a transmission circle occurring in the area, which could serve as an indicator of the risk of exposure of human populations of contracting infection with the parasite. This is more so because water serves as a very important component of living for humans and animals and could also serve has a vehicle of transmission of some waterborne disease organisms such as the parasite under study.

It is also important to maintain surveillance of opportunistic parasites such as *Cryptosporidium* in fish in the study area. This would help to create awereness in immunocompromised patients especially HIV/ AIDs patients of the need to prevent such infection which could arise from fish processing and consumption as the disease may present life threatening conplications in them. Consequently, this may serve to enlighten

the general public on the need to take necessary hygienic measures when handling fish. In addition, there is paucity of information on the occurrence of *Cryptosporidium* in fish in Nigeria generally. Results obtained from this work in Zaria may help to establish a better understanding on the prevalence of *Cryptosporidium* in *Clarias* spp in relation to management practices by the farmers and practices of consumers.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the Study

To determine the occurrence of *Cryptosporidium* oocysts in *Clarias gariepinus* and their water habitats in Zaria, Kaduna State, Nigeria

1.4.2 Objectives of the Study

The objectives of the study were to determine:

1. the prevalence of *Cryptosporidium* in *Clarias gariepinus* and their habitats (water) in Zaria, Kaduna State.
2. the effect of the physico-chemical parameters of the water habitats of catfish on the occurrence of *Cryptosporidium* in water sampled in Zaria, Kaduna State.
3. the association between the prevalence of *Cryptosporidium* in *Clarias gariepinus* and sex, weight and length of catfish as well as fish management practices such frequency of pond water replacement, source of water, type of pond and method of dead catfish disposal in Zaria, Kaduna State.

1.5 Research Questions

1. What is the prevalence of *Cryptosporidium* in *Clarias gariepinus* and their habitats (water), in Zaria, Kaduna State?

2. Do the physico-chemical parameters of the water habitats of catfish have any effect on the occurrence *Cryptosporidium* in water sampled in Zaria, Kaduna State?
3. Is there any association between the prevalence of *Cryptosporidium* in *Clarias gariepinus* and sex, weight and length of catfish as well as fish management practices such frequency of pond water replacement, source of water, type of pond and method of dead catfish disposal in Zaria, Kaduna State?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Introduction

Cryptosporidium is a zoonotic apicomplexan parasite. It infests the gastrointestinal tract of humans and animals causing severe diarrhea illness. First discovered by Ernest Edward Tyzzer in the year 1907 in the gastric mucosa of mice, In the following decade, cryptosporidiosis emerged worldwide as a common cause of severe or life-threatening infection in immunocompromised patients, especially those with AIDS, and of acute, self-limiting gastroenteritis in otherwise healthy subjects, especially children. *Cryptosporidium* remained largely unrecognized as a human pathogen until the first reported case in 1976 in an immunocompetent child; it has become a parasite of medical and veterinary importance that causes gastroenteritis in a variety of vertebrate hosts. This organism produces environmentally resistant oocysts, which are excreted in the feces of infected individuals. *Cryptosporidium* is recognized worldwide as a waterborne pathogen, and the species *Cryptosporidium parvum* is the major cause of cryptosporidial infectins in humans and livestock (Anane, 2011).

Cryptosporidium was first described in the early 20th century; *Cryptosporidium muris* and *C. parvum* were the first species described (Tyzzer, 1912). The veterinary importance of *Cryptosporidium* species was highlighted by the associations of *C. meleagridis* with morbidity and mortality in turkeys in the 1950s and of *C. parvum* with bipovine diarrhea in the early 1970s. *Cryptosporidium parvum* is now regarded as an economically important cause of neonatal diarrhea in calves and lambs. Another species, *Cryptosporidium baileyi,*

is recognized as an important cause of respiratory disease in poultry and game birds (Bouzid *et al.,* 2013).

Cryptosporidiosis characteristically results in watery diarrhea that may sometimes be profused and prolonged. Diarrhea and abdominal pain are generally the symptoms which cause patients to seek medical attention, leading to a laboratory diagnosis of cryptosporidiosis. Other clinical features include nausea, vomiting, and low-grade fever. Occasionally, nonspecific symptoms such as myalgia, weakness, malaise, headache, and anorexia occur. The severity, persistence, and ultimate outcome of the infection are typically dependent on a variety of parasite characteristics and host factors. Host factors include both the immune status and frequency of exposure of the infected individual; however, little is known regarding the pathogenic characteristics of *Cryptosporidium* spp. The severity of a *Cryptosporidium* infection can vary from an asymptomatic shedding of oocysts to a severe and life-threatening disease (Anane, 2011). Immunocompetent individuals experience a transient self-limiting illness (up to 2 to 3 weeks). However, for immunocompromised patients, cryptosporidiosis can be a critical illness with persistent symptoms leading to dehydration and wasting and was associated with significant mortality rates (Bouzid *et al.,* 2013). In addition, *Cryptosporidium* infection can cause atypical manifestations in immunocompromised patients, such as atypical gastrointestinal disease, biliary tract disease, respiratory tract disease, and pancreatitis (Hunter and Nichols, 2002).

These apicomplexan parasites infect the microvillus border of the gastrointestinal epithelium of a wide range of vertebrate hosts and present a wide spectrum of clinical

symptons in infected individuals. The pathogenicity of *Cryptosporidium* varies with the species of parasites involved and the type, age, and immune status of the host. In many animals, *Cryptosporidium* infections are not associated with clinical signs or are associated with only acute, self-limiting illness. In some animals, such as reptiles infected with *Cryptosporidium serpentis* or individuals who are immunosuppressed, the infection is frequently chronic and can eventually be lethal. Due to wide host range of *Cryptosporidium* spp. and the ubiquitous presence of oocysts in the environment, humans and animals can acquire the infections through several transmission routes. In pediatric and elderly populations, especially in day care centers and nursing homes, person-to-person transmission probably plays a major role in the spread of *Cryptosporidium* infections. In rural areas, zoonotic infections via direct contact with farm animals have been reported many times, but the relative importance of direct zoonotic transmission of cryptosporidiosis is not entirely clear (Bouzid *et al.,* 2013). Numerous outbreaks of cryptosporidiosis due to contaminated food or water (drinking or recreational) have been reported in several industrialized nations, and studies have sometimes identified water as a major route of *Cryptosporidium* transmission in areas where the disease is endemic (WHO, 2006).

2.2 Taxonomy of *Cryptosporidium*

Over 100 years have passed since Ernest Edward Tyzzer first made his observations on the genus *Cryptosporidium*. *Cryptosporidium* was so named because of the absence of sporocysts within the oocysts, a characteristic of other coccidia. The first species described was *Cryptosporidium muris*, from the gastric glands of laboratory mice (Tyzzer, 1907). All members of the genus *Cryptosporidium* are intracellular. Tyzzer later published a complete

description of this parasite giving a clear account of the life cycle. Subsequently, he described a second species, also from laboratory mice. (Tyzzer, 1910, 1912). Following Tyzzer's discovery of *Cryptosporidium*, this species were intially confused with other apicomplexan genera, especially members of the coccidian genus *Sarcocystis* (Anane, 2011).

Once the true differences between *Cryptosporidium* and *Sarcocystis* were clearly recongzied, the concept of strict host specificity was initially adopted for naming *Cryptosporidium* species. Cross-transmission studies subsequently demonstrated that *Cryptosporidium* isolates from different animals can also be transmitted from one host species to another. This ended the practice of naming species based on host origin and brougth about synonymization of new *Cryptosporidium* species as *C. parvum*. Recently, molecular characterizations of *Cryptosporidium* have helped to clarify the confusion in *Cryptosporidium* taxonomy and validate the existence of multiple species in each vertebrate class (Anane, 2011).

conceqently, several new species of *Cryptosporidium* have also been named, *C. andersoni* from cattle, *C. canis* from dogs, *C. molnari* from fish and *C. hominis* from humans (Alvarez-Pellitero and Sitja-Bobadilla, 2002; Xiao *et al.*, 2003). Human cryptosporidiosis was initially attributed to *C. parvum* but genetic analyses indicated that two distint species are responsibly for most human infections: *C. parvum*, (formly *Crypsporidium parvum* "bovine" or "cattle" genotype; genotype 2 or C) is considered zoonotic and *C. hominis* (formly *C. parvum* "human "genotype; genotype 1 or H) as the anthroponotic species

(Morgan *et al.,* 2000). These observations, subsequently confirmed by many studies, were significant in proving that humans also play a distinct role in transmission cycles, one comprising ruminants and humans and the other exclusively comprising humans (Anane, 2011). Some isolates, once identified *C. parvum*, or genotypes of *C. parvum* have been elevated to species level, these include; *Cryptosporidium hominis*, *C. bovis*, *Cryptosporidium suis.* Other isolates of *C.parvum*, as well as those of *C. canis*, *C. galli*, and *C. muris*, have been differentiated at the molecular level and designated as genotypes or subgenotypes. During the last two decades species definition and identification of genus has been changing, with the discovery and addition of new species and subtypes. Initially, there were 14 commonly accepted species of this genus (Xiao *et al.,* 2001b, Fayer *et al.,* 2004), few years later, Xiao and Fayer (2008) reported 16 valid species of *Cryptosporidium* species with over 40 genotypes, this was later was updated to 19 (Chalmers and Giles, 2010), and subsequently 26 species were described and validated on the basis of morphological, biological and molecular data with over 60 *Cryptosporidium* genotypes (Anane, 2011, Ryan *et al*., 2014), at the time of writing this report, the number of species of *Cryptosporidium* regarded as valid were at least 32 has described by the Office International des Epizootics (OIE, 2016), with a number of new animal hosts. The number of *Cryptosporidium* species infecting humans is an ever-changing situation. Nearly 20 species and genotypes from a large varity of vertabrate hosts have been reported in human which include: *Cryptosporidium andersoni*, *Cryptosporidium felis*, *Cryptosporidium parvum,Cryptosporidium bovis*, *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*, *Cryptosporidium galli*, *Cryptosporidium scophthalmi*, *Cryptosporidium molnari*, *Cryptosporidium wrari*, *Cryptosporidium hominis*,

Cryptosporidium canis, *Cryptosporidium suis*, *Cryptosporidium serpentis*, *Cryptosporidium cuniculus*, *Cryptosporidium wrairi* and *Cryptosporidium muris* (Xiao and Fayer 2008, Anane, 2011). However, *C. hominis* and *C. parvum* are recognized globally as the most important *Cryptosporidium* species infecting humans (Xiao, 2010, Anane, 2011). The four basic requirements for the naming of *Cryptosporidium* species (Chalmers, 2008, Xiao, 2010, Anane 2011) are:

1. Morphometric study of oocysts and, if possible, sporozoites.
2. Multi-locus genetic characterisation by nucleotide sequence analysis of well studied genes or non-coding regions.
3. Demonstration of natural and, if possible, experimental, host specificity.
4. Compliance with the International Code of Zoonotic Nomenclature (Anane, 2011).

2.3 Molecular Epidemiology of Cryptosporidiosis

Molecular identification of a parasite is of great importance in various aspects of human and veterinary parasitology, most importantly in taxonomy, diagnosis and treatment. This process usually involves the precise identification such parasite at the species and or genotype level (Ruecker *et al.,* 2012). Different tools have been developed to detect and differentiate *Cryptosporidium* at the species/genotype and subtype levels. These tools have been used more in characterizing the transmission of *Cryptosporidium* species in humans and animals (Xiao, 2010). The genotyping techniques is used to differentiate *Cryptosporidium* species infecting humans from those infecting animals, it can also track sources of contamination, as well as evaluate the risk of infection for both human and animals (Xiao and Feng, 2008). The most commonly used technique in genotyping of

Cryptosporidium in humans, animals and water samples is the Small subunit (SSU) rRNA-based tools.

Molecular investigations have shown that the vast majority of human cases are caused by C. hominis and C. parvum. Molecular analyses have also revealed that other species, including C. meleagridis, C. felis, C. canis, C. suis, C. muris can infect humans and may be linked to clinical disease, not only in immunocompromised but also in immunocompetent individuals (Caccio and Widmer, 2015). A three years review of original studies on *Cryptosporidium* genotyping reported by Xiao *et al.* (2010) revealed the use of SSU rRNA tools in 100 (86%) of 116 publications, particularly, a polymerase chain reaction – restriction fragment length polymorphism (PCR – RFLP) tool that targets an 830 base pairs (bp) fragment of the gene and uses restriction enzyme from *Streptomyces* spp (Sspl) and restriction enzyme from *Vibrio* spp (VspI) for genotyping was reported in 70 (60%) 0f publications.

PCR is known to have the advantage of improved sensitivity and specificity of which 97 – 100% and 100 % have been found respectively for the sensitivity and specificity of PCR (Omoruyi *et al.,* 2014). However, these techniques have limited applicability due to the high costs, high technical expertise and infrastructure needs involved. Yet still, PCR has been found useful for the differentiation of *Cryptosporidium* spp. as well as the specific diagnosis of species / genotype (Samra, 2013). Moreso, results of molecular epidemiologic studies have led to better appreciation of the public health importance of *Cryptosporidium* species/genotypes in various animals including piscine species. It has also been used in identification of new genotypes as well as species in fish and other animal host.

2.4 Epidemiology of Cryptosporidiosis in Humans and Animals

Cryptosporidiosis has a worldwide distribution, but the prevalence of infection is assumed to be higher in developing countries (5% to >10%) than in developed countries (<1% to 3%) (Putignani and Menichella, 2010). However, in many developed and developing countries, surveillance systems for routine detection of cryptosporidiosis are not in place, and few studies have been conducted to estimate how prevalence can vary over time (Nichols, 2008), as the epidermiology of the disease has been dynamically changing over the past decade from a rare, largely asymtomatic infection to an acute enteric disease of humans and animals (O'Donoghue, 1995). These species of parasites have been estimated to infect about 500 million people annually in developing nations. *Cryptosporidium* has been listed as the leading cause of diarrhoea in children in developing countries (Dillingham *et al*., 2002) and has been found in over 170 different host species worldwide (O'Donoghue, 1995).

The distribution of the major *Cryptosporidium* species infecting humans varies geographically. Xiao and Ryan (2008) reported that *C. parvum* and *C. hominis* are responsible for >90 % of human cases of cryptosporidiosis in most areas. In the United Kingdom, in other European countries and in New Zealand, *C. parvum* is responsible for slightly more infections than *C. hominis* (Xiao, 2010). In the Middle East, *C. parvum* is the dominant species in humans. In contrast, *C. hominis* is responsible for more infections than *C. parvum* in the United States, Australia, China and Japan, as well as in most developing countries (Anane, 2011). Notably, the prevalence of *C. meleagridis* can be as high as that of *C. parvum* in certain areas of the world (Cama *et al.*, 2008). Major differences in

transmission routes may account for the observed differences in the distribution of *Cryptosporidium* species (Xiao, 2010).

Epidemiological studies involving pets, farm animals and accidental infection of veterinary workers have been used to confirm the zoonotic transmission of these parasites (Reese *et al.,* 2004). Several studies have indicated that children less than 2 years of age are more likely to be infected than older children (Kahn *et al.,* 2004, Gatie *et al*., 2006). Kahn *et al.* (2004) reported that sexual practices that imply oral-anal contact with diarrhoea or HIV/AIDs patients, has led to high exposure to *Cryptosporidium* infection worldwide. Such practices are also considered as risk factors that predisposes to cryptosporidiosis in addition to poor hygeine practices among risk individuals (children, pregnant women, the elderly, etc). This is evident from high prevalences of infection recorded from such populations in some regions.

Other factors that contribute to the wide spread of cryptosporidiosis are related to the parasite. These include the lack of maturation period (sporulation) required outside the host to become infective as with other coccidian species, secondly, the ability of the oocyst to survive for a long period in the environment. These oocysts may survive for 2 to 6 months in the environment (Carey *et al.,* 2004). Again, the thick walled oocysts excreted in faeces of an infected host are fully sporulated (O'Donoghue, 1995), and very hardy to environmental pressures, they are well resistant posing significant problem for water providers. Oocysts are readily infectious once excreted in the environment and a new cycle is initiated following ingestion by a suspectible host (O'Donoghue, 1995).

Epidermiological studies have demonstrated that millions of *Cryptosporidium* oocysts can be released in a bowel movement from an infected animal or person (El-madawy *et al*., 2010). Shedding begins once symptom manifests (diarrhoea) and can last for weeks after symptoms have stopped. Generally, all species of this genus are similarly small, with low host specificity and resistant to routine disinfection procedures which are all in addition to lack of effective treatment (El-madawy *et al.,* 2010). Other contributing factors to the spread of *Cryptosporidium* infection is the parasite's unique location within the host cell, sequestered between the cell cytoplasm and cell membrane, its ability to autoinfect, and its innate antimicrobial resistance (Anane, 2011).

2.5 Cryptosporidiosis in Animals

Cryptosporidiosis is considered to be an infection of socioeconomic and public health importance (OIE, 2008). Some *Cryptosporidium* species appear to be restricted to particular types of hosts, whilst others have broad host range, including man, farm and wild animals such species are considered zoonotic (Thompson *et al*., 2008). Different prevalence and infection rates have been reported globally from different animal hosts. In cattle, infection by *Cryptosporidium* species was first reported in 1971 (Chalmers and Giles, 2010; Peter, 2014). Four species have been isolated from cattle (*C parvum*, *C andersoni*, *C bovis*, and *C ryanae*) (Chalmers and Giles, 2010; Maikai *et al.,* 2011). *Cryptosporidium andersoni* infects the abomasum of older cattle; *C bovis* and *C. ryanae* are cattle adapted. *Cryptosporidium parvum* is a common cause of calf diarrhea, and cryptosporidial oocysts have been detected in the feces of 70% of 1- to 3-weeks-old dairy calves (Peter, 2014). Fathia (1993) found that the infection rate in diarrheic calves less than 1 month was 65.5% while it was 33.3% in calves aged 1-2 months. Infection can be spread

from animal to animal by the fecal-oral route, usually when animals are housed together in an overcrowded environment, contamination of udder and water supplies by feaces are other common sources of transmission in livestock (Alaa *et al.*, 2015). Infection can be detected as early as 5 days of age, with the greatest proportion of calves excreting organisms between days 9 and 14. Many reports associate infection in calves with diarrhea occurring at 5–15 days of age (Peter, 2014). Cattle have been considered to be a primary reservoir for *Cryptosporidium* oocysts for zoonotic *C. parvum* (Chalmers and Giles, 2010).

Adult sheep and cattle can act as asymptomatic carriers shedding small numbers of oocysts to the environment. This was shown to increase in number in the perinatal period and contribute to maintaining the infection between parturition periods (Hill *et al.,* 1990; Xiao and Herd, 1994, Alaa *et al.,* 2015). These animals could be a risk factor via environmental contamination from their manure being spread on farmland or their grazing on watersheds. On farms, transmission of *Cryptosporidium* speceies can result from ingestion of contaminated food or water, by direct transmission from host to host, or through other indirect means (Alaa *et al.,* 2015).

High mortality due to cryptosporidiosis has been reported in calves, even in the absence of other enteropathogens. Infections are mainly concentrated in the distal small intestine but lesions were also found in the caecum and colon and occasionally in the duodenum. The pathological findings associated with *Cryptosporidium* are a mild to moderate villous atrophy, villous fusion, and changes in the surface epithelium. In addition, infiltration of mononuclear cells and neutrophils in the lamina propria has been reported (Angus, 1990). The economic losses due to cryptosporidial infections of neonatal calves are related to

diarrhoea and its treatment: dehydratation, growth retardation and to a lesser extent mortality (Alaa *et al*., 2015). Diarrhoeic problems of calves demand special care which would usually require feeding of electrolyte solutions, intravenous fluid therapy, drug administration, hygienic measures, etc. which arecostly as well as labour and time consuming (De-Graff *et al.*, 1999).

Cryptosporidium parvum is also a cause of infection in young lambs and goats. In sheep and goats, Cryptosporidial infection was first described in Australia in 1- to 3-week-old lambs and 2 weeks old kid with diarrhoea *Cryptosporidium* has been listed as an important cause of in neonatal diarrhoea syndrome in this domestic species and is currently associated with high morbidity rates and, depending on environmental conditions and the presence of other intestinal pathogens, mortality (Angus, 1990; De-Graff *et al.*, 1999). Diarrhea can result from a monoinfection but more commonly is associated with mixed infections. Infection can be associated with severe outbreaks of diarrhea, with high case fatality rates in lambs 4–10 days and in goat kids 5–21 days old (Peter, 2014). Economic losses associated with infection in small ruminants are similar to those of cattle.

Cryptosporidial infection in pigs is seen over a wider age range than in ruminants and has been seen in pigs from 1 week old through market age. Naturally occurring cryptosporidiosis in pigs has been described worldwide (Kim, 1990). Most infections are asymptomatic, and the organism does not appear to be an important enteric pathogen in pigs (Peter, 2014), although it may contribute to postweaning malabsorptive diarrhea (De-Graff *et al.*, 1999). Digestive disorders are the predominant cause of mortality reported in

commercial rabbits. It has been reported by Peters (1988) that the parasite mainly affects weaned rabbits of 4 to 8 weeks of age. Peters (1988) also reported a prevalence of 2-11% in weaned diarrhoeic Belgian rabbits. In general, field outbreaks of cryptosporidiosis in suckling rabbits are rarely detected and in weaned rabbits the parasite causes only subclinical enteritis (De-Graff *et al.,* 1999).

Avian cryptosporidiosis is an emerging health problem in poultry, associated with respiratory disease in chicken turkeys and quails. (De- Graff *et al.,* 1999). First reports of a *Cryptosporidium* sp. in avian species were from Tyzzer (1929). He found a parasite in the caecal epithelium of chickens with structural similarities to *C. parvum,* found in mice. Infection by *Cryptosporidium* spp. has been detected in over 30 species of birds including domesticated chickens, turkeys, ducks, geese, quails, pheasants, peacocks, and a wide variety of wild and captive birds (O'Donoghue, 1995 Fayer *et al.,* 1997, Bamaiyi *et al.,* 2013). Naturally occurring cryptosporidiosis in chickens usually manifests as respiratory disease, and occasionally as intestinal or renal disease. Symptoms usually include depression, anorexia, emaciation, coughing, sneezing; dyspnoea, as well as increased mortality are most often associated with respiratory cryptosporidiosis. The economic losses associated with this disease are due to poor flock performance. This is usually as a result of growth retardation and increased consumption index in addition to mortality (De-Graff *et al*., 1999).

Cryptosporidial infection in foals appears less prevalent and is seen at a later age than in ruminants, with excretion rates peaking at 5–8 weeks old. Infection is not usually detected

in yearlings or adults (Peter, 2014). Most studies indicate that cryptosporidiosis is not a common disease in foals; infections in immunocompetent foals are usually subclinical however, persistent clinical infections are seen in Arabian foals with inherited combined immunodeficiency (Peter, 2014). Most studies indicate that *Cryptosporidium* also infects picine species in their wild and cultured environment (Maikai, 2012; Atawodi and Bichi, 2013; Certad *et al.,* 2015; Costa *et al.,* 2016), also in both salt and fresh water species as well as in aquatic species such as carps (Yang *et al.,* 2016) however, infection in these species are not well described.

2.6 Waterborne Cryptosporidiosis

Waterborne infectious diseases are a globally emerging public health issue. Contamination of water bodies have led to various community outbreaks which have highlighted the importance of intestinal protozoa in public health. Among these important pathogens are *Giardia duodenalis*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Isospora belli*, *Microsporidia* and, of greater relevance, *Cryptosporidium* (Karanis *et al.,* 2007). Oocysts and cysts of these parasites have been reported in the aquatic environments throughout the world. They have been found in most surface waters, where their concentration is related to the level of faecal pollution or human use of the water (LeChevallier *et al.,* 1991). The oocysts of *Cryptosporidium* are environmentally robust, very persistent in water and extremely resistant to the disinfectants commonly used in drinking-water treatment. (Karanis *et al.,* 2007). These characteristics, coupled with the low numbers of oocysts required for an infection place these organisms among the most critical pathogens in the production of safe drinking-water from surface water (Rose, 1997). Ground waters that mix with surface water or other sources of contamination (e.g. surface run-off) may contain low

levels of *Cryptosporidium* and give rise to waterborne illness Filtration of such waters is essential to the production of safe drinking-water as treatment by disinfection alone offers no protection against *Cryptosporidium* (Anane, 2011).

Waterborne transmission of the oocysts and outbreaks of cryptosporidiosis either through drinking water or recreational use is well documented and have been listed by several authors (Rose, 1997; Oppenheimer *et al.*, 2000; Walter *et al.*, 2002; Anane, 2011). Waterborne *Cryptosporidium* outbreaks have occurred in both large and small communities, with the largest outbreak occurring in Milwaukee, Wisconsin in 1993, affecting an estimated 403,000 people (Anane, 2011). Such outbreaks have caused major disruption to residents, businesses, and government. Infection with the *Cryptosporidium* organism may also have contributed to the premature deaths of immunosuppressed individuals in these outbreaks (Anane, 2011). The ubiquitous presence of *Cryptosporidium* spp. in the aquatic environment is explained by the large number of hosts, the extremely high number of oocysts shed by these hosts, and the remarkable stability of oocysts (Yoder and Beach, 2007). Thus, water represents a very important vehicle of infection for the population, and waterborne cryptosporidiosis is a serious public health concern, particularly for populations at risk of severe infection (pregnant women, children, HIV-positive and transplanted patients) (Caccio and Widmer, 2014). During the last decade about 71 *Cryptosporidium*-linked outbreaks have been described, and 40 (56.3 %) appear to be correlated to waterborne transmission (Caccio and Widmer, 2014). Geographically, the outbreaks seem to be concentrated in the USA, Canada, Australia and Europe, especially in the UK and Ireland, and affect both adults and children (Putignani and

Menichella, 2010; Chalmers, 2012). Surveillance data has revealed the presence of *Cryptosporidium* spp. in the entire water treatment system which represents an unacceptable health risk, particularly for at risk populations (pregnant women, children, HIV-positive and transplanted patients) (Caccio and Widmer, 2014). This suggests that focus ought to be placed on prevention of human and animal waste contamination especially in authorized recreational waters. Remarkably, cryptosporidiosis is the most frequently reported gastrointestinal illness in outbreaks associated with treated (disinfected) recreational water venues in USA (Yoder and Beach, 2007).

2.6.1 Contributing factors for the spread of water borne cryptosporidiosis

Waterborne outbreaks of cryptosporidiosis have been documented in countries around the world. Between the years 1986 and 1996, a total of 16 cryptosporidiosis outbreaks associated with drinking water was reported in Europe, majority in England and Wales, and 14 in North America, the majority in the USA. Two outbreaks were reported in Japan. Most of the *Cryptosporidium* recreational water outbreaks have been documented in the USA and the UK (Caccio and Widmer, 2014). Rainfall was a strong variable in drinking water outbreaks and fecal accidents in recreational outbreaks (Joan *et al.*, 2006). Several factors have been known to play distinctive rolls in the spread of water borne cryptosporidiosis. These factors include; biological, environmental, climatic and community factors. The biological factors include excretion rates, zoonotic transmission and environmental stability and infectivity of the oocyst. High production and long-term excretion (weeks) of the infectious oocyst stage by infected hosts (ranging from 10^6 to 10^{11} per gram of feces) have been documented, thus contributing to high loading to environmental pollution of waters (Fayer *et al.*, 1997a). Moreso, the oocyst is extremely

resistant once shed into the environment and can survive in water for weeks (Joan *et al.*, 2006).

The water source is one of the key environmental factors. The type of land use activities contributing feaces for example shows that waters receiving cattle and sewage discharges have 10–100-fold greater concentrations of oocysts. The occurrence of *Cryptosporidium* oocysts in surface waters has been reported in 4–100% of the samples examined at levels depending on the impact from sewage and animals (Lisle and Rose, 1995). Surface waters used for drinking supplies have been shown to be more susceptible to contamination. However, groundwater once thought to be a more protected source has shown between 9.5 and 22% of samples positive for *Cryptosporidium*, although at low concentrations (Joan *et al*., 2006). The climatic factors include temperature; lower temperatures enhance survival of oocysts, and rainfall, increased precipitation is associated with increased concentrations of oocysts (Alherholt *et al.,* 1999). Finally, community factors include watershed management (i.e. the presence of combined sewer overflows, leading to raw sewage discharges with levels as high a 1.3×10^4 oocysts/100 l) and type of water treatment (filtration and disinfection) (Rose, 1997, Joan *et al.,* 2006). Climatic factors have been hypothesized as a major factor in the transmission of *Cryptosporidium*. Anane, (2011) reported that data on drinking water outbreaks in the USA from 1971 to 1994 from all infectious agents demonstrated a distinct seasonality, a spatial clustering in key watersheds and a statistical association with extreme precipitation (Rose *et al.,* 2000). This suggests that in key watersheds, by virtue of the land use, fecal contaminants from both human sewage and animal wastes are transported to waterways and drinking water supplies by

precipitation events (Joan *et al.,* 2006). Correlations between increased rainfall and increased *Cryptosporidium* oocyst concentrations in river water have been reported (Atherholt *et al.,* 1999).

The Milwaukee outbreak in 1993 (USA) as well as Oxford/Swindon *Cryptosporidium* outbreak were associated with rain fall events and storm run offs (Rose, 1997). There has also been significant association between the rainy season and cryptosporidiosis cases in parts of India, South Africa and Mexico (Natt *et al.,* 1999). Finally, the incidences of infection in the animal or human population, the excretion of oocysts in certain watersheds are also important factors contributing to waterborne disease. The type of animal waste handling and sewage treatment, as well as the type of disposal will influence the likelihood of oocysts ending up in the environment. The size of the watershed, dilution and hydrology of the system as well as the type and reliability of the drinking water treatment will influence the impact of pathogens in the drinking water. Thus human, infrastructure and engineering factors also play an important role in the potential for waterborne disease, where to date; precipitation has been shown to be important in the transportation of pathogens like *Cryptosporidium* (Joan *et al.,* 2006).

2.6.2. Monitoring and detection *Cryptosporidium* oocysts in water

The most commonly used method for detection of *Cryptosporidium* oocysts in water includes filtration of large volumes of water (10–1000 l), followed by the processing (washing) of the filter to recover the captured material, then centrifugation, clarification (density gradients or more often immunomagnetic separation, IMS) and finally microscopic screening of the sample after staining with monoclonal antibodies tagged with

fluorescein isothiocyanate (using epifluorescent microscopy) (IFA methods) (Anane, 2011).

2.7. Transmission of Cryptosporidiosis

Transmission occurs through faeco-oral route, following contact with *Cryptosporidium* oocysts either directly or indirectly which is facilitated by a relatively low infectious dose and resistance of the parasite oocyst stage to commonly used disinfection techniques. This infection is usually spread in a number of ways: person to person, animal to animal, foodborne, waterborne and most importantly zoonotic route. Cryptosporidiosis is now the most common cause of waterborne disease in the world (Xiao & Ryan, 2004). Significant outbreaks involving contamination of water bodies with sewage and agricultural wastes have been reported. In rural areas, zoonotic infections via direct contact with farm animals have been reported many times (Anane, 2011).

Oocysts excreted by infected hosts contaminate the environment and initiate infections when ingested by susceptible hosts. Some oocysts are thought to be auto-infective and may excyst in the same host. Most infections are transmitted by fomites between individuals and animals held in close confinement, such as in child day-care centers, hospitals, zoos, and intensive animal rearing facilities. Numerous studies have also indicated the possibility of person-to person transmission of cryptosporidiosis occurring within homes, health care centers as well as urban settings with high population densities (USEPA, 2001). Food-borne transmission has also been recorded involving consumption of contaminated foods such as vegetables, milk, fruits, meat product, etc, probably attributable to contaminated water used in processing and production of such food products (Anane, 2011), more so,

the use of manure as a source of fertilizer in farming increases the potentials of such food borne transmissions.

2.8 Morphology and Life Cycle *Cryptosporidium*

Cryptosporidium is classified taxonomically as a sporozoa. This is because the oocyst releases four sporozoites. These sporozoites are held within a tough two-layered wall in the oocyst. The oocyst is small, measuring 4-6 µm in diameter. It is spherical-to-ovoid in shape (Nurul and Baha, 2013). The life cycle of *Cryptosporidium* is completed in a single host which involves both asexual and sexual stages. Basically, the cycle involves six major developmental stages as described by Tzipori and Ward (2002), these are: excystation, merogony, gametogony, fertilization and zygote development, formation of environmentally resistant oocyst wall formation, and sporogony. The oocyst is the exogenous stage; it is environmentally stable, able to survive through routine wastewater treatment and is resistant to inactivation by commonly used drinking water disinfectants (CDC, 2010).

Infection of *Cryptosporidium* in a new host results from the ingestion of these oocysts. Once ingested, the oocyst opens (excystation) in the gastrointestinal tract, this phenominum has been reported to be triggered by a number of factors including: interaction of stomach acid, carbon dioxide, body temperature, pancreatic enzymes and bile salts (Alaa *et al.,* 2015). This results in rupturing and release of the sporozoites in the small intestine (ileum) (O'Donoghue, 1995).

The sporozoites have naked nucleus chromosomes that consist of 10.1-10.4 million base pairs of DNA (Bankier *et al.,* 2003). They are motile 5- by 1-µm forms that adhere to and

invade the absorptive epithelial cells lining the gastrointestinal tract. After attachment and discharge of the organellar contents, the parasite focally disrupts the microvilli of the host cell giving it way to slide into the host cell and envelop itself in the cell membrane of the host (Bankier *et al.*, 2003).At this point, the parasite quickly internalizes within an intracellular but extracytoplasmic compartment separated from the cytoplasm by an electron-dense layer that appears to be predominantly of host origin (Barta and Thompson, 2006). The parasite is located within a closed compartment of the host cell plasmalemma, described as the parasitophorous vacuole (Thompson *et al.*, 2005), where it intracellularly develops to trophozoite as well as protected from the hostile gut environment and is supplied with energy and nutrients by the host cell through a feeder organelle. This intracellular extra-cytoplasmic location is unique to the coccidian parasites (Tzipori and ward, 2002).

In these cells, trophozoites undergo proliferation by merogony to form meronts, Cell division occurs by endopolygeny where multiple daughter cells are formed by internal budding within the mother meront (Alaa *et al.*,2015) Most studies performed on *Cryptosporidium* spp. have described sequential development involving two types of meronts (Fayer *et al*., 1997b; Spano and Crisanti, 2000); Type I meronts form 8 merozoites which are liberated from the parasitophorus vacuole when matured, they invade other epithelial cells where they undergo another cycle of type I merogony or develop into type II meronts and completes the asexual stage of the life cycle. These type II meronts form four merozoites which differentiate into micro and macrogamonts which are sexual reproductive stages (gametogony) (O'Donoghue, 1995). Upon fertilization of the macrogamont by the microgamont, zygote is developed, which ultimately develops into an

oocyst. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host, and the thin-walled oocyst, which is primarily involved in autoinfection.Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission (Anane, 2011). Thin-walled oocysts re-circulate in the intestinal tract causing autoinfection (O'Donoghue 1995; Hijjaw *et al*., 2004; Nurul and Baha, 2013). This phenomenon may explain the mechanism of persistent infection in AIDS patients in the absence of subsequent oocyst exposure (Tzipori and Ward, 2002).

2.9 Laboratory Diagnosis of Cryptosporidiosis

Several techniques have been employed for the diagnosis of cryptosporidial infections for both clinical and epidemiological purposes, these include histology and ultra-structural examination of biopsy material for lifecycle stages, endogenous (tissue) stages can be shown by light and electron microscopical techniques in biopsy and necropsy tissues (Casemore, 1991), detection of oocysts in faeces, as well as detection of antigens and DNA in humans and animals (Casemore, 1991; Xiao and Fayer, 2008; Samra, 2013; Omoruyi *et al*., 2014). Faecal specimens are often preserved in 10% formalin, sodium acetate-acetic acid formalin (SAF), polyvinyl alcohol (PVA) fixatives however, PVA preserved specimens are limited to some particular staining mathods. Oocyst viability is retained following storage in 2.5% potassium dichromate or at 4ºC. Storage in formalin for extended periods should be avoided if molecular analyses are required (Xiao and Fayer, 2008). Oocysts recovered from stool are easily identified using differential staining methods such as safranin-methylene blue stain, modified Kinyoun's acid fast method,

Ziehl-Neelsen and stain staining method which stains oocysts red and counterstain the background (Omoruyi, 2010).

Immunological based assays include: the enzyme-linked immunosorbent assay (ELISA). This is also known as enzyme immunoassay (EIA), and all its derived tests such as the falcon assay screening tests ELISA (FAST-ELISA), the dot –ELISA. Other assays include monoclonal antibody immunofluorescence (IFAT): this often shows the characteristic suture line on the surface of the oocyst. Although the use of indirect immunofluorescence antibody (IFAT) has been described for routine stool examination, this is not widely practiced, partly because it is expensive (Casemore, 1991), the haemagglutination test, complement fixation (CF) test, and immune-blotting and rapid diagnostic tests (Omoruyi, 2010). Molecular based techniques are often required for species identification including genome detection (PCR amplification of the 18S rRNA gene) (Areeshi *et al.*, 2007). Many fluids and tissues may be submitted for analysis, this could include stools, sputum, bile, mucoid secretions, and tissue biopsies, however, stool specimen are most preferred especially for enteropathogenic species and genotypes (Xiao and Fayer, 2008). All specimens are amenable to staining, antigen, detection and molecular based methods (Xiao and Fayer, 2008).

2.9.1 Concentration of oocysts

Faeces from patients with acute cryptosporidiosis do not usually require concentration to detect oocysts, although the numbers of oocysts excreted can fluctuate during the course of the infection (Casemore, 1991). Oocysts are more readily detected in concentrations from watery specimens than from formed stools. In specimens containing small numbers of

oocysts, sensitivity can be increased by employing a concentration method followed by staining (Xiao and Fayer, 2008). Concentration of specimens may sometimes be indicated in the management of immunocompromised patients with a previous history of unexplained diarrhoea. Such patients can occasionally experience remission and subsequent recrudescence of cryptosporidiosis (Casemore, 1991).

2.9.1.1. Oocyst concentration by centrifugation

Oocysts settle more rapidly if the stool suspension is subjected to centrifugation. For human stools, after adding formalin for fixation and preservation, the level of detection can be increased by adding ether or ettyl acetate to remove fats and oils. After centrifugation, a fatty plug will be visible at the interface of the the two liquids. The ether layer, fatty plugs, and formalin layer is discarded and the pellet retained for examination (Xiao and Fayer, 2008, OIE, 2016). This process is reported to be more efficient than sedimentation by gravity, as a smaller faecal sample is sufficient for examination. The 10% formalin and ether are bactericidial (Xiao and Fayer, 2008). Many modifications to this procedure have been advocated, however, less distortion of protozoan cysts occurs with this method than zinc sulphate flotation as well as achieves a concentration of 15-50 folds, dependent on the parasite type, and provides good concentrate of protozoan cysts and helminth eggs, which are diagnostically satisfactory (Xiao and Fayer, 2008).

2.9.1.2. Oocyst concentration by floatation

The flotation principle utilizes a liquid suspending medium that is denser than the oocysts to be concentrated. The density of intact, viable *Cryptosporidium parvum* oocysts is

approximately 1.05g/m^3 (settling velocity = 0.0018m/h) (Smith, 1992). Therefore when mixed with flotation fluid, the oocysts rise to the surface and can be skimmed out of the surface film and detected using the chosen method. For a flotation fluid to be useful in diagnostics, when morphology and morphometry are the critical factors, the suspending medium must not be heavier than the object to be floated as well as not produce shrinkage sufficient to render the object undiagnosable (Smith, 1992). Sucrose flotation, zinc sulphate floatation and saturated salt flotation methods are all suitable for concentration of *Cryptosporidium* oocysts.

2.9.2. Conventional staining methods for detection of *Cryptosporidium* oocysts

Acid-fast staining methods, with or without stool concentration, are most frequently used in clinical laboratories. For greatest sensitivity and specificity, immonofluorecence microscopy is the method of choice. Molecular methods are mainly a research tool. The most common staining methods include modified Ziehl Nelsen (mZN); auramine-phenol (AP), Wright- Giemsa, and safranin-methylene blue and fluorescein isothiocyanate (FITC) labelled monoclonal antibody. Both mZN and AP are effective for detection of *Cryptosporidium* oocysts in faeces (Casemore, 1991). Modified Ziehl- Nelsen (mZN) stained slides should be screened under the ×40 objective lens and putative oocysts confirmed and measured under the ×100 objective lens using a bright –field microscope with ×10 eyepiece (Casemore, 1985). Auramine-phenol (AP) stained slides require to be read using an epi-fluorescence microscope equipped with a fluorescene isothiocyanate (FITC) filter set (excitation 490 nm; emission 510 nm). A UV filter set (excitation 355 nm, emission 450 nm) can assist in visualizing AP-stained sporozoites. The AP-stained slides can be screened under the ×20 objective lens and oocysts with typical morphology can be

confirmed under the ×40 objective lens. The ×100 objective lens must be used for all morphometric measurements. Any AP-stained oocyst visualized under either the FITC or UV filters can be measured by slowly increasing the voltage (light intensity) of the bright – field light source so that both fluorescent and bright-field images can be seen concurrently (Casemore, 1991).

2.9.2.1. Modified Ziehl Neelsen (mZN) staining method

Modified Ziehl-Neelsen staining was introduced for staining cryptosporidial oocysts by veterinary workers who had found that *Cryptosporidium* was associated with scouring of calves. It was first used to detect *Cryptosporidium* oocysts in feaces in 1981 (Henriksen and Pohlenz, 1981). It has been described has the most widely used method because of its simplicity and low cost (CDC, 2013). It basically involves making a thin smear on a clean glass slide which is air -dried and fixed in methanol for 3 minutes. The fixed smear is then flooded in cold carbol-fushsin and allowed to stain 15 minutes after which, it is rinsed thoroughly in tap water. This is then decolourised in 1% acid- alcohol for 10 – 15 seconds, rinsed in tap water, counter stained with malachite green or methylene blue for 30 seconds, rinsed again in tap water, and allowed to air –dry. The stained slide is then examined for the presence of oocysts under the × 40 objective lens and confirmed under the oil immersion lens. *Cryptosporidium* oocysts stain pink – red on a blue – pale green background depending on whether it is methylene blue or malachite green used for the counter stain. The degree and proportion of staining varies with individual oocysts (OIE, 2016). In particular, infections that are resolving can have colourless oocyst "ghosts", mature oocysts may discernible sporozoites (up to 4) (CDC, 2013).

2.9.2.2 *Auramine – phenol (AP) staining method*

Auramine – phenol (AP) staining is very reliable, either when used for staining mycobacteria or in a method developed for staining oocysts which is also widely used (Casemore, 1991). This method also requires fixing an air – dried smear in absolute methanol for 3 minutes. This is immersed in auramin- phenol stain for 10 minutes and rinsed in tap water to remove excess stain. The slide is then decolourised with 3% acid alcohol for 5 minutes, counter stained in 0.1% potassium permanganate for 30 seconds and air – dried at room tempreture. The dried slide is examined for the the presence of oocysts using the epiflurescence microscope equipped with fluorescein isothiocyanate (FITC) or UV filters under the ×20 objective lens and confirmed under the ×40 objective lens. *Cryptosporidium* oocysts usually appear ring or ovoid shaped and exhibit a characteristically bright apple – green fluorescence against a dark background (OIE, 2016)

2.9.3 Measurement of oocysts

Measurement of putative oocyst is essential to ensure that they fall within the accepted range of standard parameters for the species in question (Casemore, 1991). At the light microscope level, measurement of objects <1 mm is achieved by means of a stage micrometer used in conjunction with an eye-piece micrometer. Objects are measured in Systeme International (SI) units, and the standard unit of measurement for conventional microscopy is the micron (μ = 0.001 mm) (OIE, 2008). The stage micrometer consists of a 76 × 26 mm glass slide that has a millimeter scale, graduated in microns permanently mounted on it. The eye-piece micrometer is a disc of transparent glass or plastic bearing a graduated scale, which is placed in one of the eye-pieces of a binocular microscope. The

scale is usually 1 cm in length and is subdivided into millimeter intervals (Casemore, 1991).

When the microscope is focused on the object to be measured, both the scale on the eye-piece micrometer and the image of the object are seen simultaneously in focus. The standard scale on the stage micrometer is usually 1 or 2 mm. When measurements are to be made, the appropriate objective lens, which is dependent on the magnification required, is chosen, and the number of divisions corresponding to the length or breadth of the image of the object is read on the scale of the eye-piece micrometer. The observed measurement is translated into real length (which corresponds to the number of eye-piece micrometer divisions representing the chosen parameter to be measured) by substituting the stage micrometer for the object and determining the number of divisions on the eye-piece micrometer corresponding to a definite number of divisions of the millimeter scale on the stage micrometer, under the same magnification (OIE, 2008).

2.10 Prevention and Control of Cryptosporidiosis

The prevention and control of cryptosporidiosis has been found difficult due to the lack of effective drugs for the treatment of the infection and because *Cryptosporidium* oocysts are resistant to many chemical disinfectants (Anane, 2011), therefore, control measures are aimed at reducing or preventing oocyst transmission which include identification of the source of infection, isolation of infected individuals, maintaining high standards of hygiene, proper effluent disposal and disinfection of contaminated surfaces. In establishments such as farms, hospitals, daycare centers, laboratories and in homes, contacts with potential sources of infection should be minimized (Omoruyi, 2010).

Since good hygiene practices have been stated as the best method of preventing cryptosporidiosis, avoiding unsafe water source, unboiled water as well as improperly uncooked foods such as fish, and improperly washed fruits and vegetables is expected espercially for the immunocompromised. Individuals with weakened immune system are advised to boil drinking water for 1 minute, or filter drinking water with devices that remove particles 1 μm and larger, or use bottled drinking water, especially water obtained from underground sources (eg. springs or wells), which are less likely to be contaminated by *Cryptosporidium* (Anane, 2011). Individuals should avoid sexual practices that can result in hand or mouth exposure to stool, avoid direct exposure to animals and avoid swallowing water when swimming. If exposure cannot be avoided, wash hands immediately after contant with such objects (Robertson and Bruno, 1997).

In farm animals, preventive hygiene measures are also the most important tools in the struggle against cryptosporidiosis, the objective being to destroy external forms of the parasite and to prevent their transmission among animals and from the environment to the host (De-Graff *et al.,* 1999). In hatcheries and husbandry practices, the destruction of oocysts in the ponds, pens and buildings used for parturition by applying moist heat, the use of abundant clean straw beds, avoidance of high stocking rates in ponds and parturition spaces can reduce infection rates in farmed fish and animals. Changing of water frequently and quarantine of weak fishes are other important means of preventing cryptosporidiosis in fish ponds. Separation of healthy and ill animals during outbreaks of diarrhoea, in addition to the administration of appropriated supplies of colostrums to neonates, all help to prevent

outbreaks of cryptosporidiosis and to minimize mortality and morbidity in infected herds (De-Graff *et al.,* 1999).

2.11 Cryptosporidiosis in Fish

Cryptosporidiosis has been reported globally in different groups of animals with much known about the epidermiology of this parasite in several host species. In fish species, little biological, epidemiological and molecular data are available, although, the parasite has been described and genetically characterized in more than 20 species of both freshwater and marine fish as reported by Certad *et al.* (2015). Alvarez-Pellitero and Sitja-Bodadilla (2002) described *Cryptosporidium molnari*; the only currently recognized species infecting fish, in sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) giving detailed histological and ultra structural features of this organism. *Cryptosporidium scophthalmi* was later detected in turbot (*Psetta maxima*, syn. *Scophthalmus maximus*) by Alvarez-Pellitero *et al*. (2004) , however, this species still lacks genetic data (Ryan *et al.,* 2014). Fish species have been reported to be infected with *Cryptosporidium* species found in other groups of vertebrates, these include; *C. parvum*, *C. hominis*, *C. scrofarum* and *C. xiaoi*. Additionally, eight *Cryptosporidium* fish genotypes, and one *Cryptosporidium* rat III-like genotype, have been described in fish (Ryan *et al.,* 2014). Recently the species *Cryptosporidium huwi was named for the piscine species from guppy* (*Poecilia reticulata*) also known as millionfish or rainbowfish, in addition to the tilapia species *Cryptosporidium cichlidis* (OIE, 2016). Moreso, a new genus, designated *Piscicryptosporidium*, has been proposed for *C. molnari* and *Cryptosporidium* –like species and genotypes affecting fish

but further genetic and biological characterisation is required to determine whether or not *Piscicryptosporidium* is a valid genus (OIE, 2016).

In fish hosts, *Cryptosporidium* fish species and genotypes are located either in the stomach or intestine, as revealed by histological analyses of researchers (Alvarez-Pellitero and Sitja-Bodadilla, 2002; Murphy *et al.,* 2009). Moreso, just as reported in other animal hosts it has also been reported that the parasite can cause clinical manifestations in fish species, such as emaciation, poor in growth rate, anorexia, whitish feces, abdominal swelling, and ascites (Alvarez-Pellitero and Sitja-Bodadilla, 2002; Alvarez-Pellitero *et al.,* 2004). An increase in the mortality rate associated with *Cryptosporidium* infection has also been reported, particularly in larval and juvenile infected fish, Murphy *et al.* (2009) reported death of about 400 larval angel fish in an aquarium system during an outbreak of cryptosporidiosis, adult angelfish were found to be relatively unaffected. Economic losses due to this infection in fish would arise from cost of treatment and feeding of infected fishes in addition to losses incurred from death of juveniles especially in farms and aquariums, losses due to poor growth only becomes evident when yields are checked at the end of growth period. Sitja-Bobadilla *et al.* (2005) found a significant correlation between the presence of the *Cryptosporidium* and both fish weight and seasonality, with the rate of infection being higher in fish weighing less than 100 grams in the spring. In addition, they also reported a relationship between the presence of the parasite and the production stage in farmed fish (Sitja- Bobadilla *et al*., 2005).

Studies on *Cryptosporidium* in fish species have shown that the epidermiology of the parasite in fish could vary significantly with geographical area. For instance, Sitjà-

Bobadilla *et al*. (2005) reported 100% prevalence of *C. scophthalmi* in juvenile turbot in Europe; In contrast, a study in Australia by Reid *et al.* (2010) found no *Cryptosporidium* isolates in freshwater fish; while a *Cryptosporidium* prevalence of 0.2% was found in wild freshwater species in Papua New Guinea (Koinari *et al.,* 2013). Certad *et al*. (2015) evaluated the prevalence of *Cryptosporidium* spp. in fish from Lake Geneva (Lac Léman) in France, with nested PCR using degenerate primers followed by sequence analysis was used, they recorded a total prevalence of (37%), distributed as follows: (87%) *C*. *parvum*, (7%) *C*. *molnari* and (7%) mixed infection (*C*. *parvum* and *C*. *molnari*). The presence of *C*. *molnari* was also detected in fish fillet (Certad *et al.,* 2015). In Isreal, *Cryptosporidium* is recognized as the common parasite of the stomach of wild and cultured cichlid fry (*Oreochromis* spp.), infection occurs with other enteric coccidiosis (Landsberg and Paperna, 1986). Meronts and gamonts of this coccidium have being reported to appear as dense spherical structures located at the brush border apices of the stomach epithelium. The attached parasite is encased within the host cell wall, whose rudimentary microvillus is visible when viewed with a scanning electron microscope (Paperna, 1987). The host microvilli are completely lacking in *Cryptosporidium* of other vertebrates. Another unique future to piscine *Cryptosporidium* is the retreat of mature zygote into the stomach mucosa or submucosa, where sporulation is completed, instead of being released into the gut lumen, as in the non-piscine forms (Makai, 2012).

2.12 Parasites of the African Catfish in Nigeria

The African catfish *Clarias gariepinus* has been found to habour many species of parasites (Wang, 2002; Moravec *et al.,* 2003). Some of these species are harmful and cause mortality

(Yu *et al.*, 1993). There are a number of parasite fauna of *C. gariepinus* in Nigeria. Earliest reports in Nigeria include that of Awachi (1966) who documented preliminary information on the parasites of fish in the Kainji reservoir. He reported that many fishes were infected. Similarly, Ukoli (1969) observed heavy parasitic infection of fish species from the same source. It has been reported that various parasites are associated with *Clarias gariepinus* both in the wild and cultured environment which cause morbidity, mortality and economic losses in aquaculture practice globally (Subashinghe, 1995). There are isolated studies of parasites in catfish in Nigeria. Oniye *et al.* (2004) examined 240 *Clarias gariepinus* for helminths in Zaria, Nigeria. They reported five species of helminth parasites including 3 cestodes, 1 nematode, and 1 acanthocephala with the following prevalences; cestodes were *Anomotaenia* sp. (2.5%), *Monobothrium* sp. (13.3%), and *Polyonchobothrium clariae* (1.7%). Nematodes; *Procamallanus laevionchus* and acanthocephalan; *Neoechinorhynchus rutili* had a prevalence of 0.8% and 0.8% respectively. In Makurdi, Benue State, Omeji *et al.* (2011) examined *C. gariepinus* for protozoan parasites and recorded a prevalence of 53.3%. They observed different protozoan parasites from different locations in the *C. gariepinus* which included *I. multifiliis* isolated from the gills and skin, *Trichodina* sp. found on skin and fin, *Icthyobodo* sp. and *Chilodonella* sp. were found on the skin, and *Cryptobia iubilans* found in the stomach and intestine. Emere and Egbe (2006) had initially reported the infection of the skin, fin, and gills in *Synodontis clarias* by these protozoan parasites in Kaduna State with the highest load of parasites reported from the gills.

Maikai (2012) examined 180 *Clarias gariepinus* for the presence of parasitic oocysts from retail outlets and backyard fish farms in Kaduna State and recorded oocyst prevalence of 21.1%. In this study, oocysts were more prevalent in retail outlets than backyard fish farms.

The study also recorded 6.6% prevalence of *Cryptosporidium* oocysts. Atawodi and Bichi (2013) conducted a study in catfish from two ponds and lakes in Zaria, Kaduna State to determine the prevalence of *Cryptosporidium* oocysts. Their study recorded a prevalence rate of 49.8% and fishes from the lakes were found to harbour more *Cryptosporidium* oocysts. Generally, it is clear that available information on picine cryptosporidiosis in Nigeria is scanty. The reason for this is not known, but could be attributed to the fact that some of the reports may be unpublished, while some may have been published in journals that are not well disseminated.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out in Zaria. Zaria is a major city in Kaduna State in Northwestern Nigeria, as well as being a Local Government Area (figure 3.1). Formly known as Zazzau, it was one of the original seven Hausa city States. The 2006 Census estimated Zaria population at 975,228 with a density of 1,400/km^2. It is located within latitudes 11^{0}04'N and 7^{0}42'E longitudes with an altitude of 675 meters above sea level and a total land area of 300 km (100sq mi) (National Population Commission, 2006). 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 adjacent Tudun-Wada neighbourhood people typically reside in traditional abode compounds. These two neighbourhoods are predominantly occupied by indigenous Hausa. The neighbourhoods of Samaru and Sabon-Gari are predominantly occupied by Nigerians of Southern origin, such as Ibo. The largest market place is in Sabon-gari. Other more recent neighbourhoods include; Danmagaji/Wusasa, PZ, Kongo, GRA-Zaria, Hanwa, Bassawa, Lowcost, Kofan-Gayan and Shika.

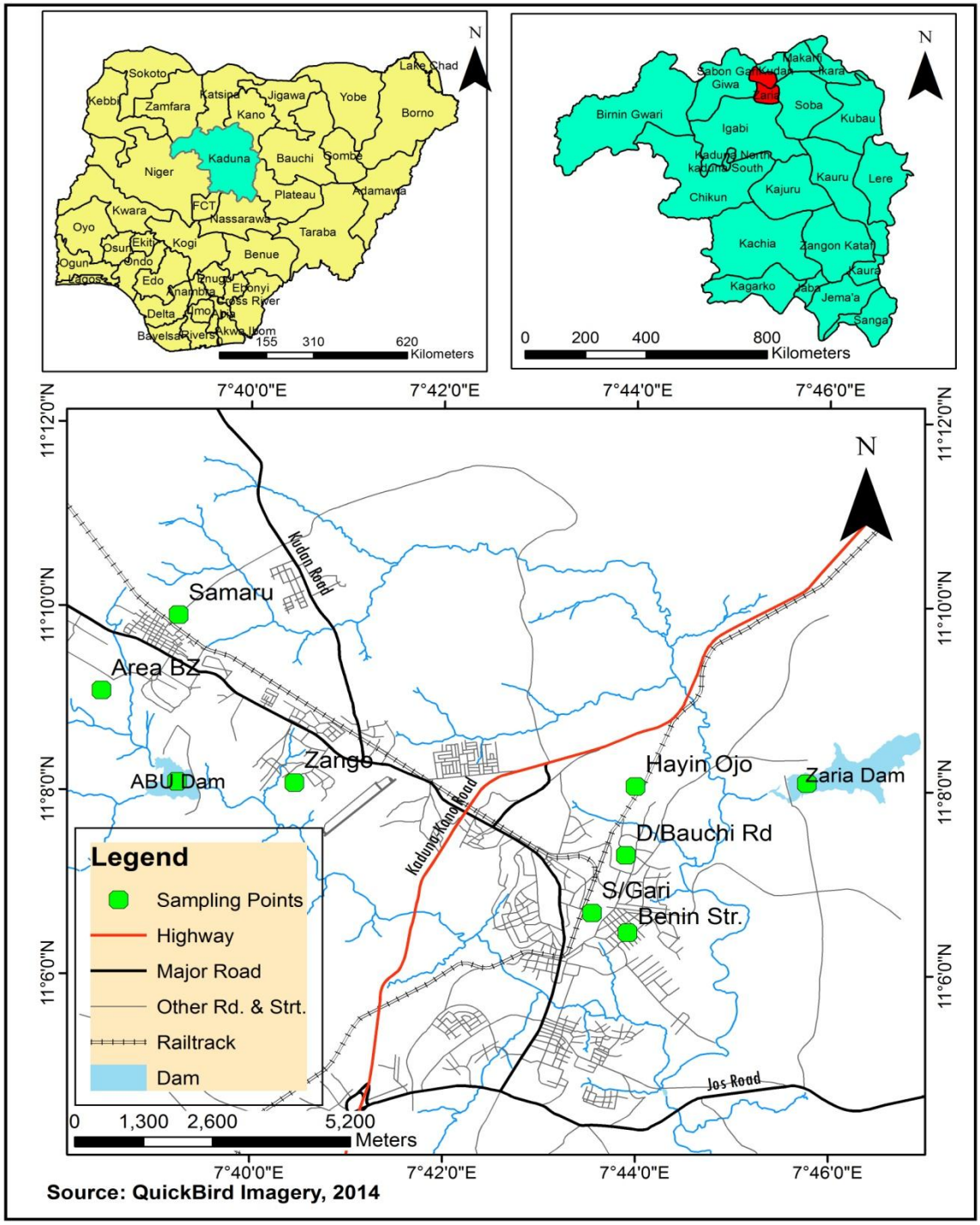

Figure 3.1: Zaria Urban Area showing sampling locations of the study.
Source: Quick Bird Imagery, 2013.

3.2 Study Design

Cross sectional study was conducted in which backyard catfish farms and dams within Zaria were selected using convenience sampling method. For the study, three backyard farms from Sabon gari and 3 from Samaru as well as ABU dam from Samaru and Zaria dam in Sabon gari were selected as shown in figure 3.2.

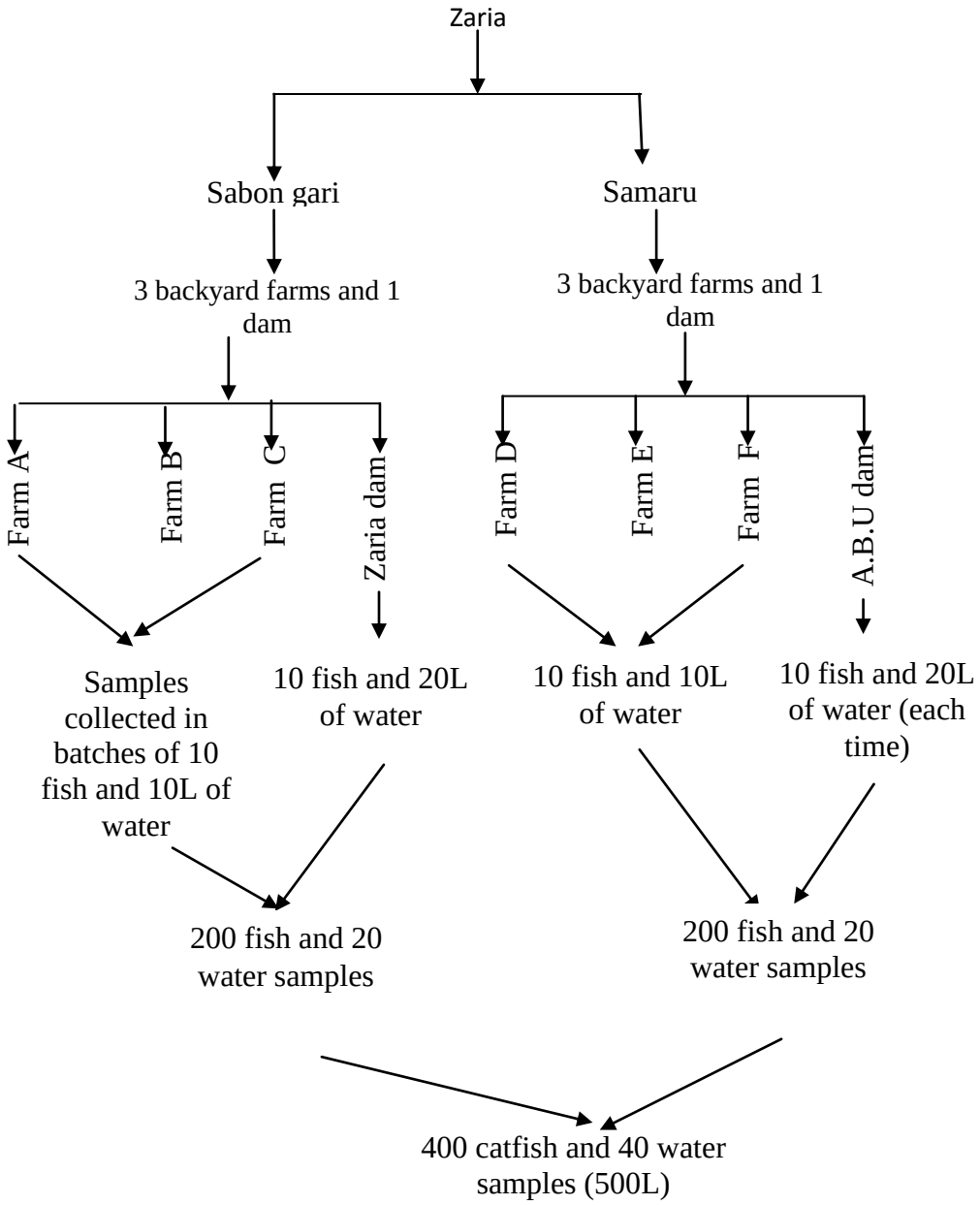

Figure 3.2: Schematic presentation of the study design

3.3 Sample Size Determination

Sample size was determined using the formula of Thrusfield (1997) at 95% confidence interval. Using the prevalence of 49.75% by Atawodi and Bichi (2013), samples were calculated using the formula below;

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

Where

N= sample size

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

P= prevalence (49.75%), Atawodi and Bichi (2013) = 0.4975

q =1-p

d= allowable error

$$N = \frac{1.96^2 (0.4975\ X\ 0.5025)}{0.05^2}$$

$$N = \frac{3.8416\ X\ 0.24999375}{0.0025}$$

N = 384.2

This was rounded up to 400 to allow collection of equal number of samples from the eight sampling units. Sample size for water was determined by dividing fish samples into batches of 10, such that, for every ten fish samples, one water sample was taken at each time of collection. Thus, a total of 400 fish samples and 40 water samples comprising of 500L were collected.

3.4 Use of Questionnaire

Structured questionnaire (appendix I) was used to obtain data on each farm sampled. Questions were centered on the following; frequency of water disposal, pond cleaning routine, type of pond and other farming practices. The questionnaire was also used to obtain information from catfish consumers on the level of awareness on diseases associated with catfish, hygiene practices as well as the socio-demographic characteristics of respondents in the study area. Questionnaire was in two sections (appendix 1). Section A was used to obtain information from consumers whilst section B was on data from each backyard farm. Questionnaire was administered prior to sample collection. A total of one hundred questionnaires were administered to catfish consumers within the study area while 6 questionnaires were administered to all the six backyard farms sampled. Number of questionnaires administered to consumers was based on availability of catfish consumers at the time of administration.

3.5 Sample Collection

3.5.1 Fish sample collection

Fish samples were purchased directly from backyard fish farms and dam. Fifty fish samples were collected from each sampling unit, 300 catfish samples were collected from 6 farms and 100 from two dams (making 400 samples). Samples were collected in batches of 10 during each collection. Sampling was done from October 2015 to May 2016. Fish samples were collected into clean plastic containers and transported to the Parasitic Zoonosis Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria for analysis.

3.5.2 Water sample collection

Water samples were collected alongside fish samples. Thirty (10 liters each alongside each batch of 10 fish samples) water samples from six backyard farms (comprising of 300L) and ten (20L each) water samples from 2 dams (comprising of 200L) making a total of 40 samples comprising of 500L was collected. Sampling location in each dam was about 1m away from the bank. From both dams and dams, water was taken slightly below the surface using a plastic sample container. All samples were processed within 48 hours of collection as described by Simmons *et al*. (2000).

3.6 Preparation of Fish Samples

3.6.1 Euthanasia of fish

Each fish was euthanized by rapid cooling (hypothermic shock). Using a hand net, the fish was transferred from the source water into cooler containing ice cold water. Care was taken to avoid direct contact of fish with ice. The fish was allowed to remain in the ice cold water for about 15 minutes following cessation of opercular movement (AVMA, 2013).

3.6.2 Determination of fish parameters

The length of the fish was determined by laying fish along a measuring board, with tip of the mouth aligned with front of the tape and the tail fin pitched close to take the whole body length of fish in centimeters. The fish body weights were taken using weighing balance in grams. The fish was sexed by checking on the anal papilla. Males have a long, conical anal papilla, while the females have a roundish opening with a central longitudinal vent (Madu *et al.*, 1999).

3.7 Fish Intestinal Sample Collection

Using a scalpel blade, each catfish was gutted dorsally just above the anterior dorsal fin and across the spinal cord, an incision was made on the ventral surface beginning from the operculum to the anal vent through which the gastrointestinal tract was excised separately into appropriately labeled tubes. The contents of each gastrointestinal tract was carefully squeezed out and analyzed immediately or preserved in 10% formalin at 4^{o}C in the refrigerator (Atawodi, and Bichi, 2013).

3.8 Laboratory Procedures

3.8.1 Determination of the physco-chemical parameters of water

3.8.1.1 Temperature and pH

Temperature of the water samples was taken using a thermometer at the point of sample collection. Also, the hydrogen ion concentration (pH) of each sample was measured using a HACH digital pH meter. The electrode probe was inserted into a glass beaker containing about 20 ml of the sample and the result was read from the screen and recorded. The pH meter was calibrated before and after each reading using freshly prepared pH buffers (7.00), (4.00) and (9.00) (Ologbosere *et al.,* 2016).

3.8.1.2 Dissolved Oxygen (DO)

In order to measure the amount of available oxygen present in the water, 250 ml DO bottles were filled to the brim with samples; care was taken to minimize contact with air. 100 ml of the sample solution was measured to which 2 drops of starch indicator was added. The resulting dark blue solution was titrated against a colourless 0.0125 M Thiosulphate solution (Ologbosere *et al.,* 2016).

3.8.1.3 Turbidity

The turbidity of the water samples were determined using a spectrophotometer in which 25 ml of the sample was dispensed into a curvette and placed in the light chamber and the absorbance was measured at a specific wavelength using distilled water as blank. The turbidity values were recorded in nepholometer turbidity unit (NTU) (APHA, 1993).

3.8.2 Concentration of water samples

Water samples were concentrated using sedimentation and centrifugation method. Prior to centrifugation, the samples were left to stand for 24 hours and the supernatant decanted. The sediment was thoroughly mixed and strained through two layers of guaze into centrifuge tubes and centrifuged for 1 minute at 650 xg (Simmons *et al*., 2000). The supernatant was decanted living a small amount of water for suspension of the sediment. Smears were made from the sediments and stained using modified Ziehl-Neelsen technique (WHO, 1991).

3.8.3 Concentration of intestinal samples

The gutted samples were concentrated using formalin-ether concentration method. Approximately 750 µl of intestinal sample was mixed in 7 ml of distill water using an applicator stick and strained into a centrifuge tube using a funnel, after which, 3ml ether was added to the formalinised solution. The centrifuge tube was then corked and shakened vigorously for 30 seconds. The cork was then removed and centrifuged at 1100 xg for 2 minutes. The tube was allowed to stand for 1 minute after which four layers become visible, the top layer consists of ether, the second is a plug of debris, third was a clear layer of formalin and the fourth was sediment. The plug of debris was loosened with an applicator stick and the liquid was poured off leaving a small amount of formalin for suspension of the sediment (OIE, 2016).

3.8.4 Staining using modified Ziehl–Neelsen method

A thin smear of both the concentrated water and intestinal samples was made on a clean glass slide and allowed to air dry. The smear was fixed in methanol for 3 minutes. The slide was then kept in a staining rack and flooded with cold carbon fuschin and allowed to stain for 15 minutes and washed with tap water. It was then decolourized with 1% hydrochloric-acid ethanol until colour ceases to flow out and rinsed in tap water. This was counterstained with methylene blue for 1 minute, and finally rinsed with tap water. It was allowed to air dry. The slide was examined microscopically using the low power objective and suspect oocysts were confirmed on the high power, objective (WHO, 1991, OIE, 2016). Positive samples appeared as bright rose-pink spherules on a blue-green background.

3.8.5 Calibration of oocyst size

The size of the oocyst was measured with the aid of an eyepiece micrometer (BX M AEITZ WETZLAR, GERMANY). This was achieved by calibrating the eyepiece micrometer from a measured scale on a micrometer glass slide known as the stage graticule / calibration scale. This calibration (determination of the micrometer value of the eyepiece) was done for each objective lens required for the measurement. This was carried out as follows; the upper lens of the required objective lens was replaced with the eyepiece micrometer and the stage graticule slide was place on the microscope stage, the eye-piece was rotated into position and the calibration scale was well focused until the scales of the eyepiece micrometer and the stage micrometer were parallel and close to each other so that the "0" line of the two scales are superimposed and clearly focused. Then, the field of the scale was carefully looked through in order to search and note other lines that were superimposed where a division of the eyepiece scale aligned exactly with a division of the calibration scale. The distance between the “0” line and the point of alignment on the graticule was measured by counting the number of intervals on the stage micrometer (usually measures from 0.1mm to 2.0 mm and each subdivision measures 0.01mm) that corresponded exactly to a whole number of divisions on the eye-piece micrometer (1 division of the eyepiece scale is calculated in µm). The distance covered by the eyepiece micrometer divisions (also known as the calibration factor / micrometer value of the eyepiece) was calculated as follows:

$$\mathbf{1000\ X}\ \frac{\text{Distance measured in mm}}{\text{Number of ocular divisions}}$$

This was carried out for each of the objective lens required (low and high power) and the micrometer values were noted (OIE, 2008).

In order to determine the size of the oocyst, the calibration scale (stage graticule) was replaced with the positive slide to be measured while the eyepiece micrometer was left intact on the calibrated objective lens. Both the eyepiece scale and the oocyst were focused sharply. The eyepiece micrometer was superimposed over the oocyst and the number of divisions for length and width was counted. The number of divisions was then translated into microns by referring to the previous calibration for the objective in use. The size of the oocyst was calculated thus:

Number of divisions covered by the oocyst × micrometer value of the eyepiece for the objective in use (Ochei and Kalhatkar, 2000).

3.9 Data Analysis

Statistical Package for Social Sciences (SPSS, version 17.0) (SPSS Inc. Chicago IL, USA) was used to analyze the data obtained. Chi square test, odds ratio and 95% confidence was used to determine the association between prevalence of *Cryptosporidium* oocysts and source of catfish (ponds, dams), management practices of the backyard farms, sex, weight and length of each fish. Student t-test was used to determine the effect of the physico – chemical parameters of the water on the occurrence of *Cryptosporidium* in water from the sampled units.Results were presented in tables and charts. Values of $P \leq 0.05$ were defined as significant. Prevalence was estimated by dividing the number of positive samples by the total number of samples multiplied by 100.

CHAPTER FOUR

4.0 RESULTS

Eighty six (21.5%) of the 400 catfish examined were positive for *Cryptosporidium* oocysts. Catfish from Zaria dam were more commonly infected 19 (38.0%) as compared to those from Ahmadu Bello University (ABU) dam 15 (30.0%). Among the backyard farms, farms A, B and E were more frequently infected 11 (22.0%) each, than farms C, D and F 7, 9 and 3 (14.0%, 18.0% and 6.0%) respectively (table 4.1).

Similarly, among the water samples from the catfish habitats, a prevalence of 7.5% was recorded. Two (6.7%) of the water samples from the backyard farms were positive whilst 1 (10.0%) from the dams was also positive for *Cryptosporidium* oocysts. There was no statistically significant differenced between the prevalence of *Cryptosporidium* and source water samples (dam or farm). (P = 1.000).

Table 4.3 shows the difference between the prevalence of *Cryptosporidium* oocysts in catfish and their water habitats. The prevalence was higer in catfish 10.75 (21.5%) than in their water habitat 3.75 (7.5%), however, there was no statistical association between the prevalence of *Cryptosporidium* in catfish and their water habitat (P = 0.0875).

Table 4.1: Prevalence of *Cryptosporidium* oocysts in catfish from backyard farms and dams within Zaria, Kaduna State, Nigeria

Sampling units	Number Examined	Number positive	Specific rate (%)
Farm A	50	11	22.0
Farm B	50	11	22.0
Farm C	50	7	14.0
Farm D	50	9	18.0
Farm E	50	11	22.0
Farm F	50	3	6.0
ABU dam	50	15	30.0
Zaria dam	50	19	38.0
Total	400	86	21.5

Table 4.2: Prevalence of *Cryptosporidium* oocysts in water from backyard catfish farms and dams within Zaria, Kaduna State, Nigeria

Source of water	Number of Units	Number examined	Number positive	Specific rate (%)	P-value
Backyard Farms	6	30	2	6.7	*P = 1.000
Dams	2	10	1	10.0	
Total	8	40	3	7.5	

*Fishers P- value

Table 4.3: Association between the prevalence of *Cryptosporidium* oocysts in catfish and their water in Zaria, Kaduna State, Nigeria

Sample type	Number examined	Number positive	Specific rate of occurrence (%)	P – value
Catfish	400	86	21.5	
Pooled water	** 40	3	7.5	* 0.0875
Total	440	89		

*Fisher's P-value

**40 water samples comprising of 500L

Table 4.4 shows the association between the prevalence of *Cryptosporidium* oocysts and source of catfish (backyard fish farms and dams). Fifty two (17.3%) of the 300 fish samples from backyard fish farms were found positive while 34 out of 100 (34.0%) of those from the wild were positive. There was a statistically significant difference (P-value = 0.0078, OR = 1.962; 95% CI on OR: 1.204< OR<3.196) between the prevalence of *Cryptosporidium* oocyst and source of catfish.

Table 4.5 shows results of the physico-chemical parameters of the water bodies (backyard catfish farms and dams) sampled. Temperature values ranged between 19.1^{o}C and 29.5^{o}C, with a mean total of 26.4 ^{o}C. Also, pH values were between 6.4 and 7.9 with mean of 7.2, minimum value for dissolved oxygen was 0.8 mg/L whilst maximum value was 3.4 mg/L and a mean total of 2.3mg/L. Turbidity ranged between 2.0 NTU and 8.4 NTU with a mean total of 5.7 NTU.

There was no statistically significant difference on the effect of physico-chemical parameters of water on occurrence of *Cryptosporidium* in water from the sampled units for tempreture, pH and dissolved oxygen ($P > 0.05$) whereas, turbidity was found to have a significant ($P < 0.05$) effect on the occurrence of *Cryptosporidium* in water from the sampled units. Mean turbidity (5.0 ± 0.18) of *Cryptosporidium* infected sampled unit was lower compared to mean (6.2 ± 0.55) of *Cryptosporidium* uninfected sampled units whilst there was no difference between the means of *Cryptosporidium* infected and uninfected sampled units of the other parameters (temperature, pH, and dissolved oxygen) (table 4.6).

Table 4.4: Association between the prevalence of *Cryptosporidium* oocysts and sources of catfish in Zaria, Kaduna State, Nigeria

Source of catfish	Number examined	Number positive	Specific rate (%)	Odds ratio (OR)	95% CI* on OR	P-value
Backyard farms	300	52	17.33	1.962	1.204 – 3.196	0.0078
Dams	100	34	34.00			
Total	400	86	21.50			

CI* - confidence interval

Table 4.5 Mean of physio-chemical parameters of water samples examined in Zaria, Kaduna State, Nigeria

Sampling units	Temperature ^{o}C	pH	Dissolved oxygen mg / L	Turbidity NTU
Backyard farm A	26.7	7.3	1.8	3.8
Backyard farm B	26.2	7.3	3	4.2
Backyard farm C	27.1	6.9	1.9	6.3
Backyard farm D	26.3	7.2	2.1	5.9
Backyard farm E	27.4	6.9	2.3	6.9
Backyard farm F	26	7.3	2.6	3.6
ABU dam	25.0	7.2	2.6	8.1
Zaria dam	26.2	7.1	2.3	7.0
Mean total	26.4	7.2	2.3	5.7

Table 4.6 Effect of the physico-chemical parameters of water on the occurrence of *Cryptosporidium* in water from the sampled units in Zaria, Kaduna State, Nigeria

Physic-chemical Parameters	Mean of infected sampled units (± S.D)	Mean of uninfected sampled units (± S.D)
Temperature (^{o}C)	26.4 ± 0.29 [a]	26.4 ± 0.40 [a]
Ph	7.2 ± 0.22[a]	7.1 ± 0.23[a]
Dissolved oxygen (mg/L)	2.4 ±0.47[a]	2.3 ± 0.18[a]
Turbidity	5.0 ± 0.18[a]	6.2 ± 0.55[b]

Means with different superscript letters are significantly ($P < 0.05$) different

Results of the morphometric measurement of oocysts recorded in this study is presented in Table 4.7, alongside the standard dimensions of the size of oocysts of *Cryptosporidium* species that corresponded to the morphometric sizes of the oocysts observed. Comparison of the dimensions of oocysts measured in this study with reported standard means/dimensions shows that three different sizes of *Cryptosporidium* oocysts were recorded in this study; 4.69 × 4.46µm, 5.05 × 4.49µm and 7.40 × 5.6µm (mean length by mean width of population). These oocysts dimensions / sizes corresponded with the size of the oocysts of the following *Cryptosporidium* species; *Cryptosporidium molnari*, *Cryptosporidium parvum* and *Cryptosporidium andersoni* respectively.

Table 4.8 shows the distribution of the oocysts sizes and corresponding *Cryptosporidium* species in both catfish and their water. In catfish, *C. molnari* oocysts were found more with a specific infection rate of 40.7%, followed by *C. parvum* oocysts with 34.9% and *C. andersoni* oocysts had the lest rate of occurrence with 5.8% specific rate. A mixed infection of *C. molnari* and *C. parvum* oocysts was also detected with a prevalence of 18.6%. Distribution of these species in the wild and cultured catfish is as follows; *C. molnari* occurrence was higher in catfish from backyard farms (59.6%) than in corresponding catfish from the wild (11.8%). Oocysts of *C. parvum* were more in catfish from the wild (50.0%) than in catfish from backyard farms (25.0%). Similarly, *Cryptosporidium andersoni* oocysts were also higher in catfish from the wild (11.8%) as compared to those from backyard farms (1.9%). Likewise, catfish with mixed infection of *C. molnari* and *C. parvum* were also higher in those from the wild (26.5%) than in corresponding catfish from backyard farms (13.5%). In the water samples, 33.3% of the

positive samples were contained *Cryptosporidium parvum* oocysts, similarly with *Cryptosporidium molnari* (33.3%) and mixed infection of both *C. parvum* and *C. molnari* (33.3%). All (100%) of the oocysts detected in water samples from the wild catfish habitat contained both *C. parvum* and *C. molnari* oocysts, 50.0% of the positive samples from backyard farms had *C. molnari* oocysts while, the rate of occurrence of *C. parvum* oocysts was also 50.0%.

Table 4.7: Morphometric measurements of 106 oocysts observed from 89 samples, in comparison with standard ranges and means of oocysts of corresponding *Cryptosporidium* species.

S/N	Corresponding *Cryptosporidium* spp	Measurement observed (±S.D of population)		Number of oocysts positive (%)	Standard range of length and width (reference)
		Mean length (µm)	Mean width(µm)		
1	*C. molnari*	4.69 (±0.07)	4.46 (±0.29)	53* (50.0)	3.25 - 5.45 x 3.02 - 5.04, mean = 4.7 x 4.5 (Alvarez - Pellitero and Sitja - Bobadilla, 2002; OIE, 2016)
2	*C. parvum*	5.05 (±0.12)	4.49 (±0.04)	48** (45.3)	4.8 - 5.6 x 4.2 x 4.8, meam = 5.0 x 4.5 (Tillay *et al.*, 1991; OIE, 2016)
3	*C. andersoni*	7.40 (± 0.47)	5.60 (±0.49)	5 (4.7)	6.0 - 8.1 x 5.0 - 6.5, mean 7.4 x5.5 (OIE, 2008; OIE, 2016)

*17 out the 53 observed were mixed infections with *C. parvum*
**17 also out of the 48 were mixed infections with *C. molnari*

Table 4.8: Distribution of oocysts and corresponding *Cryptosporidium* species in catfish and their water from backyard farms and in Zaria, Kaduna State, Nigeria

S/N	Corresponded *Cryptosporidium* Species	Samples positive from farms (%)	Samples positive from dams (%)	Total (%)
	Distribution in catfish			
1	*C. molnari*	31 (59.6)	4 (11.8)	35 (40.7)
2	*C. parvum*	13 (25.0)	17 (50.0)	30 (34.9)
3	*C. andersoni*	1 (1.9)	4 (11.8)	5 (5.8)
4	Mixed infection of *C. molnari* and *C. parvum*	7 (13.5)	9 (26.5)	16 (18.6)
	Total	52	34	86
	Distribution in water			
1	*C. molnari*	1 (50.0)	0 (0.0)	1 (33.3)
2	*C. parvum*	1 (50.0)	0 (0.0)	1 (33.3)
3	*C. molnari* and *C. parvum*	0 (0.0)	1 (100)	1 (33.3)
	Total	2	1	3

Table 4.9 shows odds ratio (OR) and 95% confidence interval (CI) on OR on the factors affecting the prevalence of *Cryptosporidium* infection in relation to fish parameters. The prevalence of *Cryptosporidium* was higher in bigger fish of 450 - 850g (28.0%) than in smaller fish of 150 - 450g (21.1%). There was no statistically significant association between the prevalence of *Cryptosporidium* oocysts and weight of catfish. (OR= 1.329; 95% CI on OR: 0.5553 < OR < 3.181; P = 0.4796). Similarly, prevalence of *Cryptosporidium* was more in lengthier fish with total length of 35 – 55cm (22.2%) as compared to less lengthy fish with total length of 15 - 35cm (21.1%). This finding was also not statistically significant (OR = 1.056; 95% CI on OR: 0.6551< OR < 1.701; P = 0.9028). Both sexes of catfish were infected with *Cryptosporidium* oocysts, but male catfish had higher rate of infection (24.4%) than females (19.2%). However, there was no statistical significance between the prevalence of *Cryptosporidium* infection and sex of catfish (OR = 0.7857, 95% CI on OR: 0.4927 < OR <1.253; P = 0.3401).

Information acquired on the managment practices of the six backyard catfish farms sampled in relation to the occurrence of *Cryptosporidium* infection is presented in Table 4.10. The prevalence was highest in farms that used concrete ponds for growing of catfish (22.0 %), followed by those that used plastic tank (20.7 %), and the least prevalence was found in catfish raised in tampolyne constructed ponds (10.0 %). The association between the rate of *Cryptosporidium* infection and type of backyard fish farm was not significantly significant (χ^2 = 0.65443, df = 2, P = 0.721) (Table 4.10).

Evaluation of the frequency of cleaning ponds used in raising of catfish in the backyard farms revealed that the rate of *Cryptosporidium* infection was highest in farms that cleaned their ponds every 3 – 4 months (quarterly) (22.0 %) followed by farms that cleaned their ponds monthly (18.0 %), while the least rate was in farms that cleaned their ponds twice a month (every two weeks) (10.0%). There was no statistically significant association between the prevalence of *Cryptosporidium* infection and frequency of cleaning ponds used in the farms. (χ^2 = 0.6543, df =2, P= 0.721) (Table 4.10).

There was a significant association between the prevalence of *Cryptosporodium* infection and frequency of pond water replacement in the farms, (χ^2 =18.94, df = 2, P = 0.0008), as farms that changed water from ponds 2-3 times per week had the highst prevalenc of *Cryptosporidium* organisms (21.0 %), followed by those that changed water 4-5 times per week (14.0 %), and the least was in farms that changed water 6-7 times per week (6.0 %) (table 4.10).

Farms that administered prophylactic treatment to their catfish recorded lower rate of *Cryptosporidium* infection (16.0 %) as compared to those that do not give any prophylaxis (20.0 %). However, there was no significant difference between the use of prophylactic administration and rate of infection (χ^2 = 0.06009, df = 1, P = 0.8064) (table 4.10).

All backyard catfish farms sampled depended on borehole or well as the source of water for raising catfish. Borehole dependent farms had slightly lower rate of *Cryptosporidium* infection (17.0 %) as compared to others that depended on well water (18.0 %), this was however not statistically significant (χ^2 = 0.033, df =1, P = 0.85) (table 4.10)

Based on the method of disposal of dead catfish found in farms, the rate of *Cryptosporidium* infections was lower in farms that are not in the practice of feeding dead catfish to other catfishes (10.0 %) as compared to those that feed dead catfish to other catfishes (21.0 %). The statistical association of this finding was significant ($\chi^2 = 4.103$, df = 1, P = 0.04) (table 4.10).

Table 4.9: Association between the prevalence of *Cryptosporidium* infection and length, weight and sex of catfish in Zaria, Kaduna State, Nigeria

Parameters	Number Examined	Number Positive	Specific Rate (%)	Odds Ratio	95% CI	P – Value
Length						
15 - 35cm	247	52	21.1	1.056	0.6551 - 1.701	0.9028
35 - 55cm	153	34	22.2			
Weight						
150 - 450g	375	79	21.1	1.329	0.5553 – 3.181	0.4796
450 – 850g	25	7	28.0			
Sex						
Male	176	43	24.4	0.7857	0.4927 - 1.253	0.3401
Female	224	43	19.2			

Table 4.10: Association between the prevalence of *Cryptosporidium* infection and management practices in backyard catfish farms in Zaria Kaduna State, Nigeria

Factors examined	Number of farms	Number of catfish examined	Number positive	Specific rate (%)	Chi square	P value and Df
Type of pond						
Concrete	1	50	11	22.0		
Plastic tank	3	150	31	20.6	0.654	0.721; 2
Tampolyne tank	2	100	10	10.0		
Pond water replacement per week						
2-3 times	4	200	42	21.0	18.94	0.0008; 2
4-5 times	1	50	7	14.0		
6-7 times	1	50	3	6.0		
Pond cleaning per month/year						
Twice monthly	2	100	10	10.0	0.654	0.721;2
Monthly	1	50	9	18.0		
Quarterly	3	150	33	22.0		
Prophylaxis						
Yes	4	200	32	16.0	0.061	0.8064; 1
No	2	100	20	20.0		
Source of water						
Borehole	4	200	34	17.0	0.033	0.85; 1
Well	2	100	18	18.0		
Disposal of dead catfish						
Feed to catfish	4	200	42	21.0	4.103	0.04; 1
Do not feed to fish	2	100	10	10.0		

A total of 100 questionnaires were administered to catfish consumers within the study area. Table 4.11 shows the demographics of these respondents, 51.0% of them resided in Samaru, 35.0% in Sabon gari, 7.0% in Zaria city, 4.0% in Tudun wada and 3.0% in other areas of Zaria. A large number of these respondents are within the age group 15 -35 years 87.0%, most of whom were males 56.0% with 82.0% having tertiary education. The least of the respondents were above 50 years of age 2.0%, 44.0% were females and 2.0% had primary education.

Assessments of the practices and preferences of respondents revealed that most catfish consumers prefer to have their catfish properly cooked (45.0%), 36.0% roasted and 19.0% like it half way cooked, also, 87.0% of all the respondents prefer the catfish fillets to the internal organs (intestines) (13.0%). With respect to the source of catfish, most of the respondents (61.0%) preferred catfish from the wild, 33.3% from farms while 6.0% have no preference (Table 4.12).

Based on the level of hygiene practices, 97.0% of respondents wash their hands after handling catfish of which 63.9% use soap or detergent, 35.1% use only water and 1.0% apply other methods in washing of their hands (such as use of lime, ash, etc), while 3.0% of all the respondents do not wash their hands after handling catfish (Table 4.12).

On the level of awareness of catfish harbouring infectious organisms, 70.0% of consumers are not aware of any diseases associated with catfish, whilst 30.0% of them are aware of one or two diseases, 14.0% of the consumers knew of the zoonotic risks associated with some of the organisms catfish harbour whilst 86.0% were unaware.

Table 4.11 Demographic information of catfish consumers in Zaria, Kaduna State, Nigeria.

Information obtained	Number of respondent	Frequency (%)
Residential area		
Sabon gari	35	35.0
Samaru	51	51.0
Zaria city	7	7.0
Tudun wada	4	4.0
Others	3	3.0
Age (years)		
15 -35	87	87.0
36 -50	11	11.0
>50	2	2.0
Gender		
Male	56	56.0
Female	44	44.0
Level of education		
Primary	2	2.0
Secondary	16	16.0
Tertiary	82	82.0

Table 4.12 Practices of catfish consumers within Zaria, Kaduna State, Nigeria.

Information obtained	Number of respondents	Frequency (%)
How do you like to eat catfish?		
Raw	0	0.00
Half cooked	19	19.0
Well cooked	45	45.0
Roasted	36	36.0
What part of catfish do you prefer?		
Fillets	87	87.0
Internal organs	13	13.0
What source of catfish do you prefer?		
Wild	61	61.0
Farms	33	33.0
No preference	6	6.0
Do you wash your hands after handling catfish?		
Yes	97	97.0
No	3	3.0
How do you wash them?		
With soap / detergent	62	63.9
Water only	34	35.1
Other methods	1	1.0

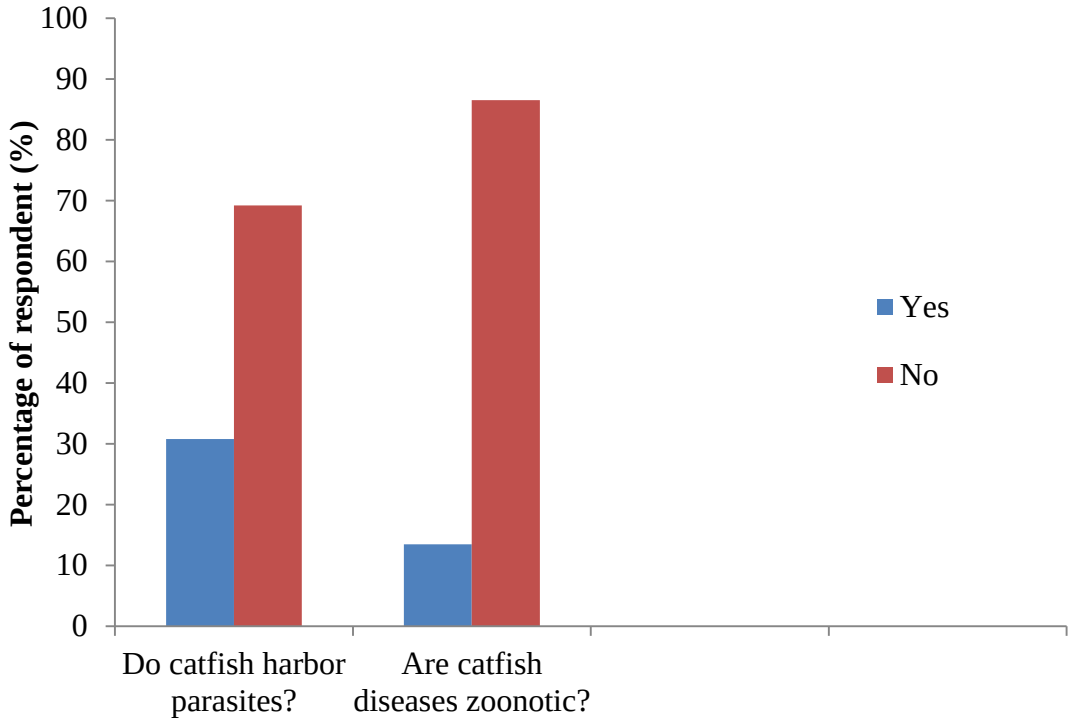

Figure 4.1 Level of awareness of consumers on catfish harboring infectious organisms

CHAPTER FIVE

5.0 DISCUSSION

In this study, the prevalence of *Cryptosporidium* was found to be relatively high, which was higher in catfish (21.5%) as compared to the prevalence in their water habitat (7.5%). The prevalence of *Cryptosporidium* in fish has been reported to vary greatly from 0.8 – 100% mostly in juveniles (Sitja-Bodadilla *et al.,* 2005; Certad *et al.,* 2015; Yang *et al.,* 2016). Similarly, the rate of detection of *Cryptosporidium* in surface waters has been reported in 4 – 100% of samples in different studies (Lisle and Rose, 1995; WHO, 2006; Anane, 2011). Thus, the prevalence found in this study fell within reported ranges of previous studies for both catfish and water. The over all prevalence of *Cryptosporidium* in catfish from backyard farms was 17.3%. This is higher compared to 10.5% reported by Atawodi and Bichi (2013) in catfish from two ponds in Zaria, Kaduna State, but similar to that of Maikai (2012) who reported 16.7% of parasitic load in catfish from backyard farms within Kaduna metropolis, Kaduna State, Nigeria.

The lower detection rate of *Cryptosporidium* in the catfish aquatic habitat was probably due to low concentrations of oocysts in the water. It has been reported that *Cryptosporidium* is found to be present in majority of surface waters, but depending on the levels of faecal pollution only few or almost all samples are found positive (WHO, 2006). The higher prevalence in catfish could probably be an indication that catfish have a relatively high susceptibility to *Cryptosporidium* parasites. More so, the parasite is thought to infect fish hosts during early stages of life when the susceptibility of the host to the parasite is increased (Sitja-Bobadilla *et al.,* 2005). Various parasites are associated with

Clarias gariepinus both in the wild and cultured environment causing high morbidity and mortality (Madu *et al.,* 1999).

The presence of *Cryptosporidium* in both catfish and their water habitat is an indication of the fact that these water bodies are contaminated with faeces whether in low or higher concentrations. This could be either as result of leakages or filtration of faecal matter from septic tanks (Atawodi and Bichi, 2013) or wastewater into the water sources used in the catfish farms or direct contamination from the farmer arising from introduction of contaminants into the ponds (Olaoye *et al.,* 2007). Sources of contamination of the dams could be directly from human and animal activities such as discharge of domestic or animal wastes into the dams, defecation by the banks, grazing activities of animals around the banks or indirectly by discharge of faecal matter in runoffs (Bern *et al.*, 2000). This finding is of public health importance especially because some of the water bodies sampled serve as source of water to a large community and the presence of *Cryptosporidium* in such water and their aquatic organisms (catfish) could lead to outbreaks of cryptosporidiosis in humans if the necessary water treatments and precautions are not put in place (Anane, 2011).

Analysis of the physio-chemical parameters of water samples showed that temperature values ranged between 19.1^{o}C and 29.5^{o}C, with a mean total of 26.4 ^{o}C. Water temperature is known to affect the infectivity of *Cryptosporidium* oocysts. Fayer *et al.* (1998), demonstrated that oocysts retained their infectivity for 1 week in water at -10°C water but remained infectious for up to 24 weeks in water at 20°C water (USEPA, 2001a). This

indicates that the temperature of the water bodies sampled were ideal for survival of oocysts even for longer periods which may contribut to the survival and spread of oocysts in catfish and their water.

Oxygen is considered one of the most requirements for survival of organisms including parasites such as *Cryptosporidium*. Although the exact level of oxygen needed for survival of *Cryptosporidium* parasites in water is not known, however, the most common cause of low dissolved oxygen in water bodies is the high concentration of micro organisms and biodegradable organic matter in water (Olaniyi, 2013). Values obtained in this study ranged between 0.8 mg/L and 3.4 mg/L, the low levels of dissolved oxygen recorded in this study may be due to activities of microorganisms in the water bodies. In general, a saturation level of at least 5 mg/L is required for optimum rests in aquaculture practices (Gupta and Gupta, 2006), values lower than this can put undue stress on the fish and increase their susceptibility to opportunistic parasites such as *Cryptosporidium.*

The pH is not known to affect the infectivity of *Cryptosporidium* oocysts in water. Smith (1992) reported no effect on survival of oocysts in water treatment processes when pH was corrected, however, some loss of viability has shown in acidic conditions below pH 4.0 and at alkaline pH above 8.0, but not at neutral pH of water such as the ones recorded in this study. However, it has been found that pH can affect fish health. For most freshwater species, pH range between 6.5 - 9.0 is said to be ideal, and the optimum pH is usually between pH 7.5 and 8.5 (Olaniyi, 2013).

Turbidity of the water samples investigated in this study was found to be low with mean of 5.7 NTU and statistically significant difference between the mean of *Cryptosporidium* infected and uninfected sampled units. It is reported that oocysts of *Cryptosporidium* can survive for up to one year in low turbidity water (USEPA, 2001b). This shows that turbidity of water bodies may affect survival oocysts in water and low values as those obtained in this study may enhance the dispersal of *Cryptosporidium* oocysts in water bodies even for a long period thereby increasing the chances of oocysts infecting potential hosts however, studies on warm water fishes have shown that fishes did not show any behavioural reaction until the turbidity approached 20,000 ppm (Gupta and Gupta 2006).Generally, fishes are most dependent on water temperature, pH, dissolved oxygen, free carbon dioxide, alkalinity and some other salts for growth and development and any change in any of these parameters may affect the growth, development and maturity of fish (Olaniyi, 2013) which could cause stress to the fish and increase their chances of being infected by infectious organisms such as *Cryptosporidium.*

Infection rates in catfish and their water habitat was higher in both catfish and water from the wild (dams). This finding is in accordance with the results of other works where prevalence of *Cryptosporidium* was high in fish species from the lakes (Atawodi and Bichi, 2013; Certad *et al.*, 2015). The high prevalence in wild catfish and water was probably due to high rate of faecal contamination of their aquatic habitats from which the fishes picked up infective oocysts. It could also be as a result of the fact that aquatic organisms in the wild cover wide distances in search of food and safety which further predisposes them to

high risk of getting infected with oocysts. Moreover, transmission and dispersion of fish parasites are facilitated by the aquatic habitat of the host (Sitja-Bobadilla *et al.,* 2005).

Though genotyping was not carried out, morphormetric measurement of oocysts revealed that oocysts of three species of *Cryptosporidium* were detected in both catfish and their aquatic habitat (4.69 × 4.46µm: *Cryptosporidium molnari,* 5.05 × 4.49µm: *Cryptosporidium parvum*, 7.40 × 5.6µm: *Cryptosporidium andersoni*- mean length by mean width of oocysts population). Currently, morphology especially oocyst measurements, represent a significant aspect of apicomplexan taxonomy which allows for identification of large numbers of genera and morphologically distinct species (Xiao *et al.,* 2003).

Cryptosporidium molnari oocysts had higher rate of occurrence in catfish and water from backyard farms. This result corresponds with the findings of Sitja-Bobadilla *et al.* (2005), where *C. molnari* was reported in cultured sea bass (*Dicentrachus labrax*) and sea bream (*Sparus aurata L*.). This finding could be an indication that the sources of *Cryptosporidium* infection in aquaculture facilities are mainly the fishes and not environmental sources, where even one infected fish could serve as the source of infection to others and infection seem to spread through water or food especially in farms where equipments are shared between ponds or facilities. Thus, early detection of infection might aid in curtailing the spread of cryptosporidiosis in aquaculture facilities.

The high rate of occurrence of *Cryptosporidium parvum* in wild catfish is of great concern especially because this species is zoonotic and large numbers of people depend on some of these water bodies. Previous works have also reported *C. parvum* in different fish species (Koinari *et al.*, 2013; Ryan and Xiao, 2014; Certad *et al.*, 2015). This could be as a result of the activities of animals around the water bodies which were evident with the presence of cattle seen grazing and frequently drinking from the banks of the dams. Also, human activities from surrounding villages around these water bodies may have led to their contamination with human faeces and domestic wastes.

Again, the high rate of occurrence of *Cryptosporidium andersoni* oocysts in catfish from the same sources further confirms the involvement of cattle in contamination of these water bodies from which the catfishes may have been infected. *Cryptosporidium andersoni* colonises the digestive glands of the abomasum of older calves and adult cattle. Infected cattle do not develop diarrhoea, but can excrete oocysts for several months (OIE, 2016). In addition, large rivers and lakes often receive both agricultural run-off and treated and untreated domestic wastewater which results in ubiquity of various *Cryptosporidium* genotypes in surface waters throughout the world (WHO, 2006). Though *C. andersoni* has not been reported in any piscine hosts, recent molecular findings have identified additional piscine genotypes as well as *Cryptosporidium parvum*, *Cryptosporidium xiaoi*, *Cryptosporidium scrofarum*, *Cryptosporidium hominis* and rat genotype III in adult and juvenile fish (Murphy *et al*., 2009; Reid *et al.*, 2010; Zanguee *et al.*, 2010; Barugahare *et al*., 2011; Koinari *et al*., 2013; Ryan and Xiao, 2014; Certad *et al*., 2015; Yang *et al*., 2015). This indicates that different piscine species could serve as hosts to a number of

Cryptosporidium species which are yet to be discovered. They could also serve as additional source of infection to humans and other animal hosts.

Mixed infection with *C. molnari* and *C.parvum* was found more frequently in wild catfish and water than in catfish and water from backyard farms. This finding is in accordance with the findings of Certad *et al.* (2015), who reported mixed infection of *C. molnari* and *C. parvum* from their molecular findings in fish species from the Lake Geneva in France. This only proves that apart from the human and animal sources of contamination, the aquatic animals also play a significant role in the transmission of *Cryptosporidium* within themselves in the aquatic habitat hence, the mixed infection and occurrence of both zoonotic and piscine type of *Cryptosporidium* in the catfish and their aquatic habitat.

Based on the catfish parameters, infection rate of *Cryptosporidium* was higher in bigger catfish both in terms of size (450 – 850g) and length (35 – 55cm). The wild catfish were lengthier (38.5%) and lighter in weight, while the cultured were heavier in weight and also lengthy indicating that the cultured ones are better fed and raised under good conditions as compared to corresponding catfish in the wild that have to search and compete for food. Bigger and lengthier catfish were more infected with *Cryptosporidium* oocysts. This was probably because bigger and lengthier catfish have high demand for food due to their size which makes them comsume more food and thus, increasing their chances of ingesting more oocysts. Also, these species of fish are omnivores and tend to feed on almost anything that comes their way (Emere and Egbe, 2006; Omeji *et al.*, 2011) increasing their chances of ingesting infectious pathogens. More so, they cannibalize on

smaller ones and pick infectious oocysts in the process (Maikai, 2012). Sitja-Bobadilla *et al.* (2005) reported that cannibalism plays a part in concentration and dispersion of cryptosporidiosis. This finding is in accordance with the results of Maikai (2012) who reported higher parasitic load in bigger catfish both in length and weight from retail outlets and farms. Also, it supports the findings of Certad *et al.* (2015) that sampled only adult fish species according to size and weight from the wild and recorded high prevalence of *Cryptosporidium* species. It is however in contrast with the results of Sitja-Bobadilla *et al.* (2005), where highest infection of values was recorded in 30g – 100g of fish class. This finding implies that catfish consumers and handlers especially the immunocompromized have to take necessary hygiene precautions when in contact with catfish.

Infection rate with *Cryptosporidium* was higher in male catfish both from the wild and cultured environment than in female, with higher rates recorded in wild species even though more females than males were sampled, but this was not significant statistically. The reason for the higher rate in males was probably due to the low immunity of the males as compared to females because in fish, size (not age) has been determined to be critical for the maturity of the immune system (Ellis, 1988; Sitja-Bobadilla *et al.,* 2005) and at a certain point of development female fishes grow bigger in size an the males due to attainment of sexual maturity and accumulation of eggs thereby making the male more susceptible to diseases. Lower susceptibility due to acquired immunity in the hosts has been reported for several parasites (Duerr *et al.,* 2003; Sitja-Bobadilla *et al.,* 2005) which could be the reason for the lower prevalence in the female catfish. Biological differences in sexes of fish host could lead to one sex being more parasitized than the other during certain

stages of development (Zanguee et al., 2010; Biu and Akorede, 2013). This finding is in contrast with the results of Rahman and Saidin (2011), who reported higher number of parasites in female fish species than males.

Based on management practices of backyard catfish farms, the highest prevalence of *Cryptosporodium* was in farms that use concrete ponds. This was probably because the surface walls of concrete ponds are not as smooth as those of plastic tanks and tampolyn ponds with waxy surfaces. Hence, the rough surfaces of concrete ponds allow for easy attachment of oocysts contained in mucus cast or excreta of fish so that even when the water is drained, they are not flushed off and consequently they survive to infect other susceptible catfish.

Farms with less pond water replacement routines (2-3 times per week) had the highest rate of *Cryptosporidium* infection, as the fishes in this case are more prone to exposure to *Cryptosporidium* oocysts accumulated in the water. Longer water replacement practices in aquaculture would mean accumulation of excreted wastes and oocysts in the water. This consequently increases the chances of catfishes coming in contact with and ingesting infective oocysts. Sitja-Bobadilla *et al.*, (2005) reported that aquaculture procedures that use recirculation systems may concentrate and facilitate oocyst dispersion. In this case, instead of recycling, the water is kept for longer days which poses a greater risk to the fish stock because of dangers of oocysts, other pathogens and ammonia accumulation which decreases level of dissolved oxygen in the water.

Farms that did not administer prophylaxis medication to catfish had higher rate of *Cryptosporidium* infection. In this case, the use of multivitamins, antihelminthhs, salt bathing were all classified as prophylaxis. Though no effective antimicrobial agent has been reported for cryptosporidiosis, but such practices would prevent the catfish from coming down with common infections which could stress the fish and weaken the immune system because fish are not as immunologically competent as higher animals (FAO, 1990). When the immune system is stressed or weak, the fish could become susceptible to opportunistic infections such as *Cryptosporidium.*

All the backyard catfish farms depended on groundwater either from hand dug wells (commonly known as wells) or boreholes (also known as drilled wells) as sources of water for the catfish. The rate of *Cryptosporidium* infection was slightly higher in farms that rely on wells (18.00%) than in borehole dependent farms (17.00%). The wells differ in depth, volume and purity of water. Drilled wells are usually machine drilled, deeper in depth and usually completed by installing properly sealed casting (vertical pipe) and well screen to prevent surface contaminants (Chibuzor, 2013), whereas, wells are usually manually dug, wider in diameter and usually with poor protection from surface contaminants (Chibuzor, 2013).

There may be more sources of contamination in hand dug wells than drilled wells, which include; runoff especially for wells that are not properly structured in lower topographical areas, most wells are freely accessed by roaming animals some of which lay down on the slabs at nights, defecating and urinating on them and eventually contaminating the water.

Moreover, the buckets used for water fetching in most cases are not washed and sometimes the rope contains sand throughout the length serving as additional sources of contamination. Once water is fetched or pumped from the wells, it is discharged directly into the ponds without any further treatments. All of these factors could have contributed to the higher prevalence of *Cryptosporidium* in farms that rely on hand dug well water.

Although, drilled wells are also not completely free of contaminants, it has been reported that runoffs could enter the aquifer through poorly sealed well casting (Nkrumah, 2013). Moreover, groundwater once thought to be a more protected source of water has shown that between 9.5 and 22% of samples were positive for *Cryptosporidium* (Joan *et al*., 2006). The involvement of runoffs which could contain human and animal faeces and domestic animals in contamination of water source used in these aquaculture facilities is evident with the dictation of *C. parvum*, and *C. andersoni* oocysts in catfish raised under intensive system. This also indicates that runoffs play a significant part in the contamination of surface and groundwater sources with different *Cryptosporidium* oocysts. The use of fine filters that could retain oocysts of *Cryptosporidium* in inlet pipes would reduce contamination of fish stock with *Cryptosporidium* arising from source water contamination in aquaculture facilities. This would consequently decrease the rate of occurrence of these parasites to a certain level there making cultured catfish safer for human handling, processing and consumption especially for the immunocompromised individuals.

Backyard catfish farms that cleaned their pond / tanks less frequently (every four months) had highest rate of *Cryptosporidium* infection. This is probably because in stocking ponds / tanks, after a certain period, sand or mud tends to accumulate at the pond / tank bottom which could harbour developmental stages of parasites or their infective stages such as oocysts of *Cryptosporidium*. If this is not regularly cleaned and disinfected, such agents could survive in soil at the bottom of ponds for long a time and eventually infect a susceptible fish. In good aquaculture practices, periodic cleaning, drying and disinfection of ponds with lime is essential for disruption of developmental stages of parasites. Moreso, occasional drying of ponds is essential. In ponds dried and subjected to freezing, even the most resistant parasites are killed, and the effect of long-lasting drying (3 weeks) of ponds under tropical conditions is almost the same (FAO, 1990). However even backyard farms that cleaned ponds / tanks regularly (every two weeks), did not have routine disinfection or pond / tank drying practice. Such practices could have attributed to the high prevalence of *Cryptosporidium* recorded in cultured catfish.

Backyard farms that fed dead catfish to others had higher rate of *Cryptosporidium* infection as compared to those that did not. This was probably because such dead fish may have died of cryptosporidiosis or any infectious diseases and feeding them to other catfish is a direct means of infecting the entire fish stock. Such practice is a great risk as all the fishes that feed on such dead fish could become infected with whatever pathogen that killed the dead one. Again, the whole pond may also become contaminated in the course of feeding on the dead fish thereby increasing the chances of other catfishes getting infected. In fish

farming, dead fish found in the pond are expected to be wrapped properly and buried deep where scavenging where animals cannot access (FAO, 1990).

The higher prevalence recorded in catfish farms in this study could be due to the differences in management practices of the backyard farms sampled. Generally, farms with more stringent and better hygiene practices such as frequent disposal / draining of water (6 -7 days /week), regular pond cleaning practices (twice / month), administration of prophylaxis, and proper disposal of dead fish had lower rates of *Cryptosporidium* infection. The most important determining factors associated with the occurrence of cryptosporidiosis in backyard catfish farms were regular and proper disposal of water and dead catfish.

A higher number of catfish consumers 45.0% preferred to have their catfish properly cooked, which is a good way of preventing fishborne parasitic infections. Also, most of them (87.0%) preferred catfish fillets. However, a matter of worry is the fact that some of these consumers preferred to have their catfish improperly cooked (19.0%), and some others preferred the internal organs (intestine) of catfish (13.0%). Consumption of improperly prepared fish enhances the transmission of fishborne parasitic diseases. More so, *Cryptosporidium* species infecting piscine hosts have been reported to be found in stomach and intestines of fish species (Alvarez-Pellitero and Sitja-Bobadilla, 2002; Certad *et al.,* 2015). Thus, such practices predispose the consumers especially the immunocompromised individuals to risk of getting infected with *Cryptosporidium* infections. Again, most of the respondents preferred catfish from the wild (61.0%) for various reasons such as being more delicious and cheaper, whilst others (33.0%) believed

cultured catfish are easily available and healthier. However, results of this study as well as those of previous works (Atawodi and Bichi, 2013) have shown that wild catfish could harbour more parasitic infections and have higher risks of transmitting different species of *Cryptosporidium* indicating that more precautions and hygiene measures are required when handling or processing such fish.

Based on hygiene practices of consumers, a significant number of them washed their hands (97.0%) after handling or processing catfish and most of them washed their hands with soap or detergent (63.9%). However, some of them also washed their hands with only water (35.1%) which is not adequate to get rid of dirt and organic matter such as fish mucus which could contain infectious pathogens. The hands must be washed properly with soap or detergent and rinsed well to remove any dirt and infectious agents.

Also, most of the respondents did not have knowledge about any diseases associated with catfish consumption (70.0%), as well as the zoonotic implication of some of these diseases (86.0%). This indicates that there is poor level of awareness among catfish consumers on the risks associated with consumption of catfish and the potentials of catfish as transmitters of zoonotic diseases. This may increase their exposure and interfare with control of *Cryptosporidium* infection in the study area.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This research work has shown the prevalence of *Cryptosporidium* in catfish and their aquatic habitats both in the wild and cultured environment in Zaria. The following findings were obtained from the study:

I. A 21.5% prevalence of *Cryptosporidium* oocysts in catfish and 7.5% in their aquatic habitat were detected with a higher prevalence in catfish (34.0%) and water (10.0%) from the wild than in the cultured catfish (17.3%) and water (6.7%).

II. The prevalence of *Cryptosporidium* was slightly higher in bigger (28.0 %) and lengthier (22.0 %) catfish as well as in male catfish (24.4 %) ($P > 0.05$) than in the smaller ones and females respectively.

III. Turbidity was found to have a significant ($P < 0.05$) effect on the occurrence of *Cryptosporidium* in water sapled within the study area while temperature, pH, and dissolved oxygen were not signicant ($P > 0.05$).

IV. *Cryptosporidium* species detected through morphometric measurements of oocysts include; *Cryptosporidium molnari* dimension (50.0 %), *Cryptosporidium parvum* dimension (45.3 %) and *Cryptosporidium andersoni dimension* (4.7%).

V. The factors associated with the prevalence of *Cryptosporidium* in catfish and their aquatic habitat in backyard farms are frequency of pond water replacement and method of disposal of dead catfish ($P < 0.05$) while frequency of cleaning ponds, administration of prophylaxis medication, type of pond, and source of water were not statistically significant ($P > 0.05$).

VI. There was a low level of awareness of consumers on diseases associated with catfish (70.0 %) and the zoonotic risks of such diseases (86.0%).

6.2 Recommendations

As a result of the findings in this study, the following recommendations are made;

I. Catfish should be washed and cooked properly prior to consumption.

II. There should be a more detailed epidemiologic data and molecular characterization of isolates in further studies.

III. Regular testing of water turbidity should be adopted by the appropriate authorities as a sentinel for monitoring of *Cryptosporidium* in water bodies.

IV. Catfish farmers should be educated and enlightened by the appropriate authorities on the need for better management and hygiene practices especially on regular replacement of water in fish ponds and proper disposal of dead fish in order to make cultured catfish safer for handling, processing and consumption.

V. There is need for public health enlightment campaign programs to educate catfish consumers and handlers that catfish could harbour organisms that could infect humans.

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APPENDIX I

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

We kindly request your cooperation to fill the form below. This questionnaire is intended to obtain information from Cat fish consumers, in Zaria. Information given out by respondents will be treated as confidential. It is part of the fulfillment of an M.Sc. thesis to be submitted to the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

SECTION A.

1. Residential Area: Sabon Gari [ ] Samaru [ ] Zaria City [ ] Tudun Wada [ ] Others _____

2. Age: 15 – 35 [ ] 36 – 50 [ ] > 50 [ ]

3. Sex: Male [ ] Female [ ]

4. Level of Education: Primary [ ] Secondary [ ] Tertiary [ ] None [ ] Others _____

5. Occupation: Civil Servant [ ] Student [ ] Trader [ ] Farmer [ ] Housewife [ ] Others:_____

6. Do you eat catfish? Yes [ ] No [ ]

7. How do you like to eat it: Raw [ ] half done [ ] Fully done [ ]

8. Do you eat catfish intestines? Yes [ ] No [ ]

9. Source of Catfish: Wild [ ] Fish pond [ ]

   (Others specify)_____

10. Why do you prefer catfish from 9 above: Sweeter [ ]
    More hygienic [ ] No preference [ ] others _____
11. How often do you eat catfish? Everyday [ ] Frequently [ ] Rarely [ ]

12. Do catfish harbour any parasites? Yes [ ] No [ ]

13. If yes to No 12 above name them: _____

14. Do catfish harbour any parasites that can be transmitted to humans?Yes [ ] NO [ ]

15. Do you wash your hands after handling catfish? Yes [ ] No [ ]

16. If yes to number 18 above, how do you wash them? With soap/detergent [ ] with water only [ ]
Others specify _____

SECTION B:

This is to obtain information from catfish farmers in Zaria, Kaduna State, Nigeria.

1. Residential Area: Sabon Gari [ ] Samaru [ ] Tudun Wada [ ] Zaria City [ ] Others specify [ ]

2. Age: 15 – 35 [ ] 36 – 50 [ ] >50 [ ]

3. Sex: Female [ ] Male [ ]

4. Why do you farm catfish? For income [ ] for pleasure [ ] For family use only [ ] Others (specify) _____

5. How long have you been growing catfish?: < 1 year [ ] ≤ 5 years [ ]
≥ 10 years [ ]

6. Do you grow other fishes? Yes [ ] No [ ]

7. If yes to number 6 above, mention the type of fish_____

8. Source of water for the fishes: River/stream [ ] Well [ ] borehole [ ] tap [ ] others specify:_____

9. Source of the young fish you grow: Wild [ ] from farms around Zaria [ ] Farms outside Zaria [ ] others specify_____

10. What type of pond do you use? Concrete [ ] earthen [ ] tanks [ ] Others _____

11. How often do you change the water? Twice a week [ ] Weekly [ ] Every two weeks [ ] Monthly [ ] others specify [ ]

12. What do you feed your fishes with? Self conpounded feed [ ] you buy conpounded feed [ ] anything available [ ]
Others specify _____

13. How many times do you feed your fish a day? Once [ ] twice [ ] thrice [ ]

14. Do you change water from one pond to another? Yes [ ] No [ ]

15. How do you discard used water from your ponds?
Flush outside [ ] give to farmers around [ ] use it on your garden or farm [ ] others specify _____

16. Do you wash your pond? Yes [ ] No [ ]

17. If yes to 17 above how often: Monthly [ ] every 3 months [ ] every 6 months [ ] other (specify) _____

18. Do wash your hands after handling your fishes? Yes [ ] No [ ]

19. Have you ever had cases of sick fishes? Yes [ ] No [ ]

20. If yes to 19 above, how do you treat them? Call a veterinarian [ ] treat by yourself [ ]

21. Have you ever had cases of dead fishes in the pond? Yes [ ] No [ ]

22. If yes to 21 above, how did you handle them? Sell [ ] eat [ ] feed to other fishes [ ] feed to dogs [ ] throw into bush [ ]
Others (specify) _____

APPENDIX II

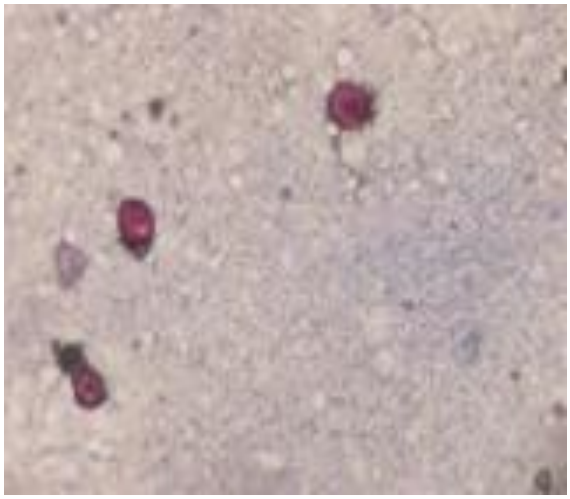

Plate I: *Cryptosporidium* parasite

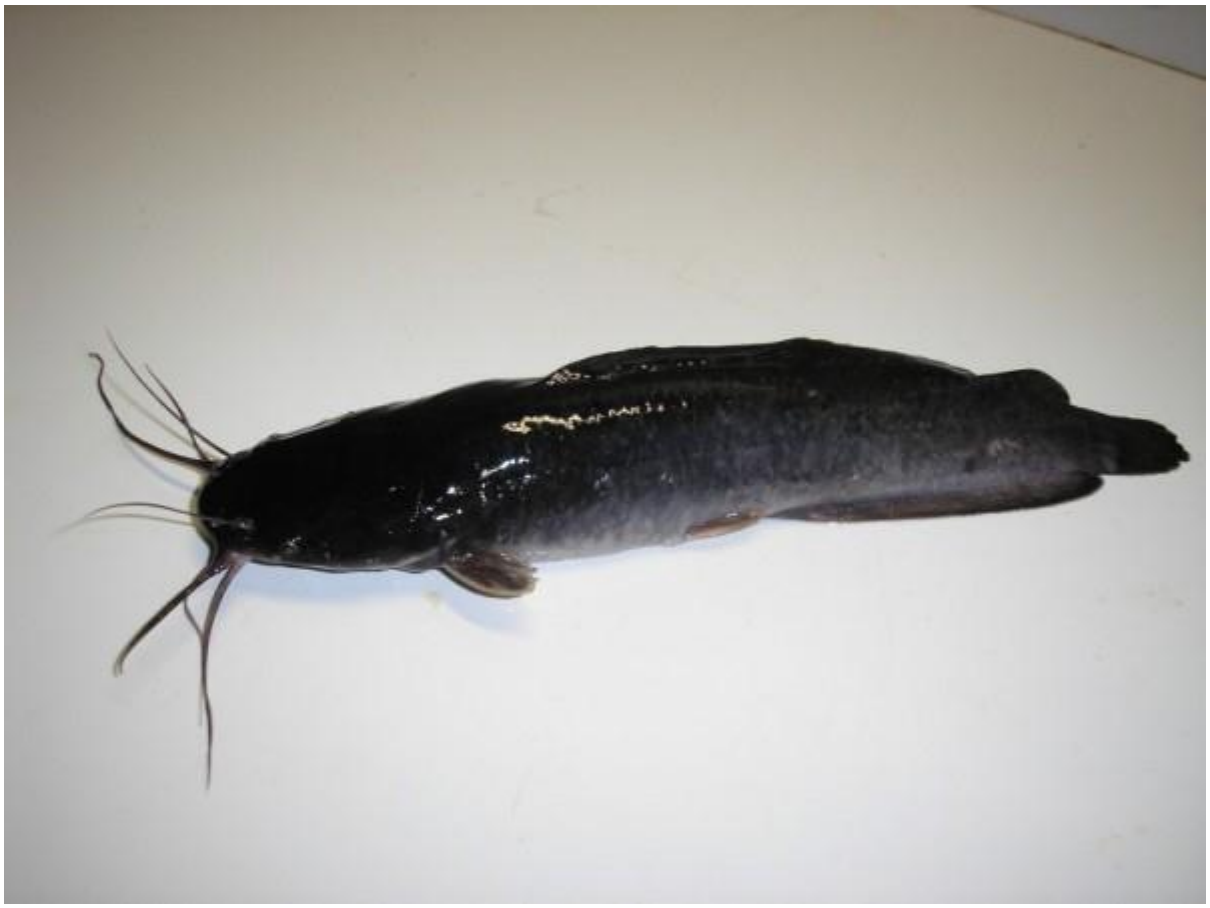

Plate II: Catfish on a measurement board

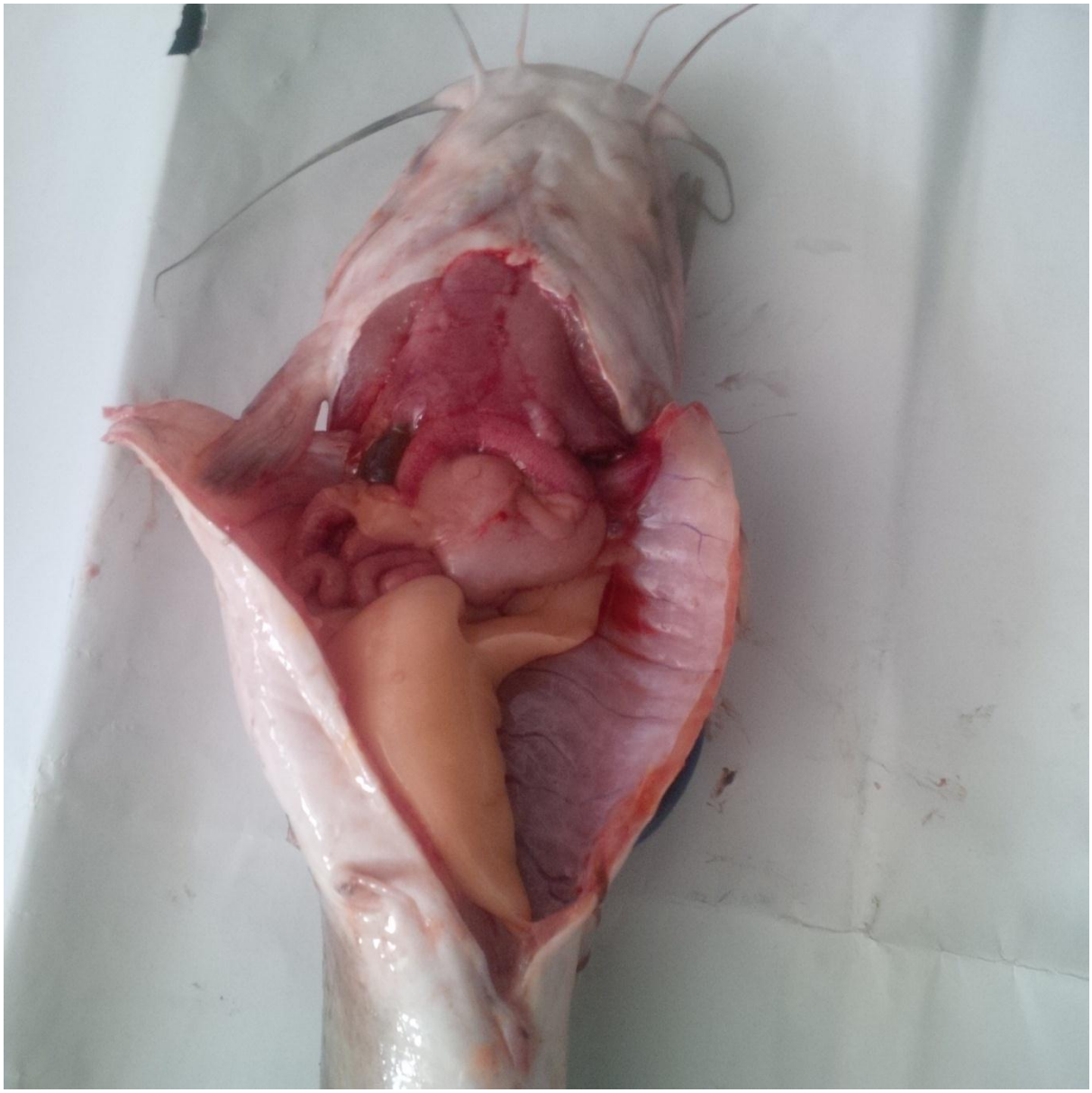

Plate III: A dissected catfish