Respiratory *Cryptosporidium* Infection in Pulmonary Tuberculosis-Suspects at Jimma University Medical Center, Jimma Southwest Ethiopia

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ABSTRACT

Background: *Respiratory Cryptosporidium infection is the presence of Cryptosporidium oocysts within upper or lower respiratory tracts in humans. It is recognized as a late-stage complication of intestinal cryptosporidiosis. The appearance of Cryptosporidium oocysts in respiratory tracts can be serious health issue, associated with significant morbidity and mortality. This study assessed the prevalence of Cryptosporidium parasite in sputum and its associated risk factors in pulmonary tuberculosis-suspects.*

Methods: *A cross-sectional study was conducted from February 1st to May 5th 2017 at Jimma University Medical Center. Data were collected using questionnaires. Sputa were screened with auramine phenol stain to detect oocysts by fluorescencent microscope. Positive sputa were re-confirmed with immunofluorescent antibody test and inoculated onto chocolate and blood agar plates. Wet mount preparations in potassium hydroxide were also done on these positive sputa. Data were entered into Epi-Data 3.1 and analyzed by SPSS version 21.0 software.*

Results*: Oocysts were detected in 9 (2.12%) sputa of 424 participants. No growth of pathogenic bacteria in cultures. Fungal elements were not observed in potassium hydroxide wet mount. Close proximity to animals, occupation, HIV and PTB infections were significantly associated with respiratory Cryptosporidium infection at p-value < 0.05.*

Conclusions*: Although the prevalence of respiratory Cryptosporidium infection looked like low, it indicated importance as a public health problem. The prevalence was significantly associated with close proximity to animals, occupation, HIV and PTB infections.*

Recommendations: *Considering the possibility of respiratory Cryptosporidium infection in pulmonary tuberculosis suspects, it is better if medical laboratory personals, who conduct auramine phenol microscopy for TB bacilli, should be familiar with the appearance of oocysts in respiratory specimens.*

Keywords: *Respiratory Cryptosporidium infection, Cryptosporidium oocysts**,** sputum, auramine-phenol, immunofluorescent anti-body test.*

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ABBREVIATIONS AND ACRONYMS

AIDS: Acquired Immunodeficiency Syndrome

AP: Auramine Phenol

FM: Fluorescent Microscopy

HIV: Human Immunodeficiency Virus

IC: Intestinal Cryptosporidiosis

IFAT: Immuno Fluorescence Antibody Test

JUMC: Jimma University Medical Center

PTB: Pulmonary Tuberculosis

PTBS: Pulmonary Tuberculosis-Suspects

RCI: Respiratory *Cryptosporidium* Infection

TB: Tuberculosis

1. INTRODUCTION

1.1. Background Information

Cryptosporidium is an obligate intracellular protozoan parasite infecting humans and a wide range of domestic and wild animals with a worldwide distribution. Cosmopolitan distribution, stability over large variation of temperature, wide host range, low infection dose, resistant to many disinfectants, lack of effective treatment and vaccine are the unique features of *Cryptosporidium* contributing to its increasing prevalence all over the world. Human cryptosporidiosis is better known as an intestinal disease in immunocompetent and immunocompromised persons. Little information exists on respiratory disease caused by *Cryptosporidium* [1, 2, 3, 4].

Respiratory *Cryptosporidium* infections (RCI) is the presence of *Cryptosporidium* oocysts in the upper and /or lower respiratory tracts of human. It is recognized as a late-stage complication of intestinal cryptosporidiosis (IC). However, most cases of RCI were reported from persons with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), solid organ-transplants, corticosteroids-therapy-taking and malnutrition, respiratory signs and symptoms are common in otherwise healthy individuals, which suggest that respiratory infection by *Cryptosporidium* species(spp) occurs in immunocompetent humans [2, 5, 6].

Sources of transmissions of RCI are multiples, involving both zoonotic and anthroponotic spreads (**figure 1**). The transmissions occur mainly by inhalation of *Cryptosporidium* oocysts via respiratory secretions, even by ingestion of oocysts during an episode of vomiting or from hematogenous dissemination in infected humans. However, hematogenous dissemination of *Cryptosporidium* oocysts is still a point of discussion; they have been also found within macrophages, leading to reduced phagocytic ability. In addition, this parasite can multiply in macrophages in vitro, which suggests that extra intestinal parasites spread via circulating phagocytes. *Cryptosporidium* oocysts are highly infectious, as few as 10 oocysts suffice to produce disease in healthy persons [3, 7, 8].

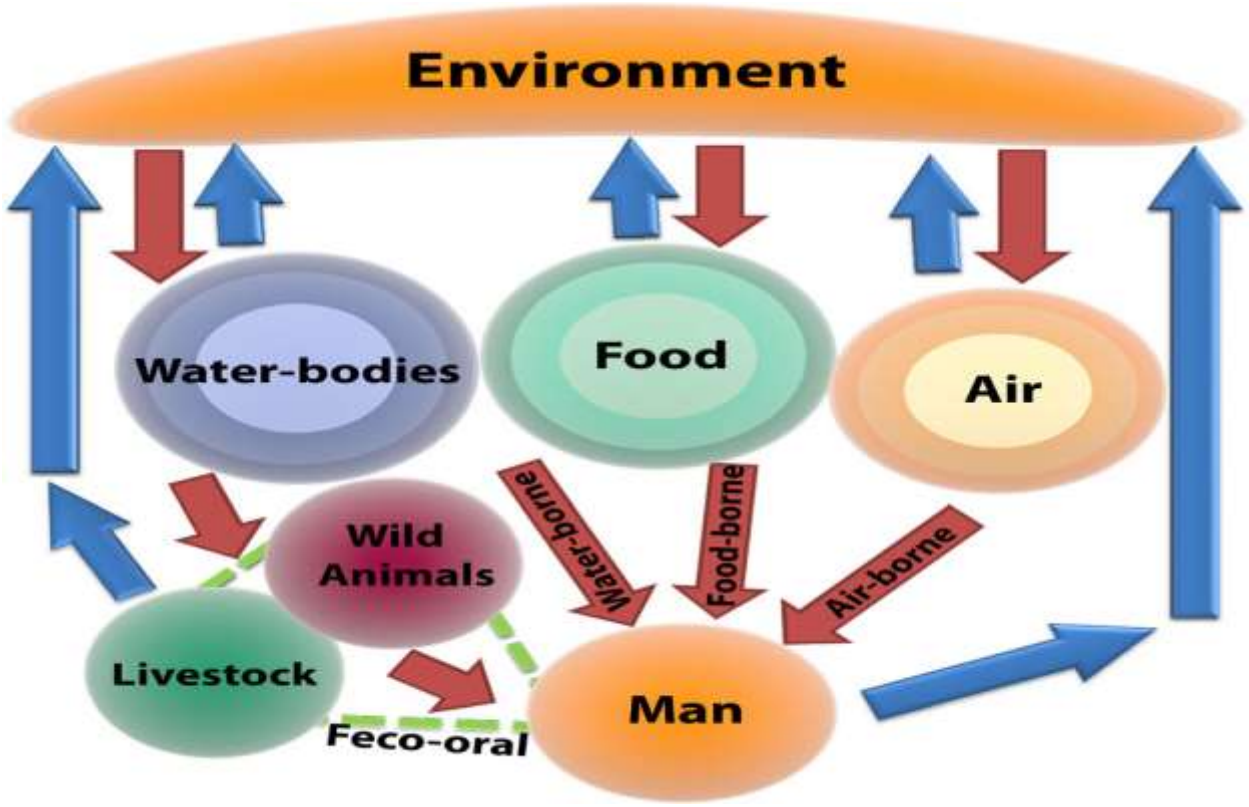

Figure 1: Routes of transmission of cryptosporidiosis, adapted from Hebatalla M. et al, 2016.

Cryptosporidium has a complex life cycle, consisting of both sexual and asexual phases. It completes its life cycle within a single host (human). Despite of nearly 27 described *Cryptosporidium* spp, *C. parvum* and *C. hominis* are responsible for the majority of cases and the most prevalent spp in humans' diseases. Both the disease and the parasite are commonly known as "Crypto."[9, 10, 11, 12].

Cryptosporidium primarily infects intestinal cells causing diarrhea ranging from asymptomatic to life-threatening. The clinical manifestations on respiratory tracts cells-infections are nonspecific and typically include difficulty in breathing, chronic cough with expectoration, low grade fever, dyspnea and thoracic pain with pneumonia being described as the most severe clinical outcome and the most common symptoms which are much similar to pulmonary tuberculosis (PTB). However, the pathogenesis of RCI has yet to be fully clarified, it brings radiological changes in respiratory tracts and lungs of many victims [13, 14, 15].

Cryptosporidium was properly recognized as a respiratory disease particularly in immunocompromised persons since the late 1980. But recent scientific evidences suggest that RCI commonly occurs in immunocompetent individuals. However, the clinical importance of RCI in immunocompetent humans may be minor; the potential for respiratory transmission is major concern. The significance of *Cryptosporidium* infection is seriously compounded by the lack of reasonable curative therapy and effective vaccine, especially in the immunocompromised and malnourished populations [6, 16, 17].

Besides, extra-intestinal cryptosporidiosis is also becoming common infection in these immunocompetent individuals, with clinical manifestations of papillary stenosis, pancreatitis and middle ear infections [13, 18, 19].

Although, microorganisms that cause respiratory infections in humans are highly prevalent and *Cryptosporidium* as respiratory disease is an emerging issue, the laboratory diagnostic techniques for perspective respiratory infections are limited or less sensitive in developing countries. Most of these respiratory infections in developing countries are often associated with significant morbidity and mortality. [20, 21, 22, 23].

Laboratory diagnosis of RCI is usually made by detection of *Cryptosporidium* oocysts, oocyst antigens, or *Cryptosporidium* DNA in sputum specimens and other respiratory tract secretions. Histologic examination of bronchial biopsies is also possible. The most commonly used method continues to be microscopic examination of sputum, tracheal aspiration or Broncho alveolar lavage by using of a variety of staining techniques like Ziehl-Neelsen, auramine phenol (AP) and immunofluorescent antibody test (IFAT) to detect *Cryptosporidium* oocysts [6, 24, 25, 26].

Respiratory *Cryptosporidium* infection is potentially concomitant to IC. On the other hands, previously conducted studies, in Ethiopia at different period of time revealed that IC is highly prevalent and endemic to Ethiopia both in human and livestock animals. Those infected livestock and pet animals could be the likely sources of zoonotic transmission to humans. Thus, based on these general evidences, it is possible to estimate the potential magnitude of prevalence of RCI in our study area [27, 28, 29, 30, 31].

1.2. Statement of the Problem

Globally, excess millions of people are burdened with chronic respiratory conditions; four million people die prematurely from chronic respiratory diseases each year and costing the global economy in excess of billion dollars. However, respiratory infection including PTB are avoidable, millions die due to the lack of access to immunizations, medications, or the inability of the health care system to provide care. Respiratory infections are still the leading cause of death in developing countries mainly in sub-Saharan African countries. Several studies have reported that 40%–50% of healthy humans all over the world, experience respiratory symptoms during IC [32, 33, 34].

Despite ubiquitous nature of cryptosporidiosis, sufficient attention has not been paid to it, the World Health Organization(WHO) in 2004 listed it among globally "neglected diseases" which have a link with poverty in most developing countries [35]. Respiratory *Cryptosporidium* infection has been recognized as new emerging diseases. Many case reports were reported from different parts of the world among both immune-deficient and immune-competent persons. It indicated that this protozoan parasite is not restricted to the gastro-intestinal tract rather exhibits extra-intestinal tropism [6, 26, 36].

It has major public health implications as infections can result from exposure to low doses of oocysts which are highly resistant to chlorination and common household disinfectants have ability to pass through physical water treatment processes, and survive long periods in the environment. Respiratory *Cryptosporidium* infection is more universal than is currently recognized. Because of diagnostic tests for *Cryptosporidium* are not routinely sought in health facilities, RCI is often under diagnosed, posing important epidemiological problems [6, 37, 38]

To our knowledge, this is the first study in entire Ethiopia to detect *Cryptosporidium* oocysts in sputum specimens and to assess the prevalence of RCI. Currently there are no documented studies at country level, might be due to lack of surveillance networks, low information and lack of appropriate laboratory diagnostic methods.

Cryptosporidium could be public health important, particularly in rural settings where humans and animals live in close proximity. Interaction with infected livestock or pet animals predisposes human to RCI. Studies conducted on both human and livestock fecal specimen in different areas of Ethiopia within different periods of time, showed high prevalence of IC in human (34.3% to 43%), in calves, (14.8%) and in lambs (2.1%)[28, 30, 39, 40, 41, 42].

It is simple fact that most Ethiopians particularly dwelling in rural areas have close proximity to animals which may have been infected with *Cryptosporidium* and may be sources of RCI. Although humans can get infection through several routes, the zoonotic transmission with respect to contact with cattle and other livestock has generated considerable interest in the epidemiology of the cryptosporidiosis [39, 43, 44, 45].

Intestinal cryptosporidiosis disseminates to the respiratory tracts with a prevalence rate of 72 % [46]. A study conducted in Uganda showed 35.4 % of children with IC developed RCI. Therefore, based on the potential concomitancy of RCI and high prevalence of IC, it is anticipated that the prevalence of RCI in Ethiopia, will be public health significant. Despite emerging parasite and its obvious relevance to public health, Knowledge about the *Cryptosporidium* as causative agent of respiratory infection remains limited [6, 47].

Respiratory *Cryptosporidium* infection generates signs and symptoms that are indistinguishable from PTB and other common respiratory diseases. In addition RCI seldom results from co-infection with other pathogens, like *Mycobacterium tuberculosis* [13, 48].

Numerous patients, who attend Jimma University Medical Center (JUMC) with productive cough, radiographic changes and symptoms of pulmonary infection, are referred for workup of chronic cough; where PTB is a likely diagnosis, but unable to detect the tubercle bacillus on sputum as expected. Based on clinical and chest radiographic findings, significant number of pulmonary tuberculosis suspects (PTBS) may receive anti-TB and antibiotics treatment without response. *Cryptosporidium* may be the one out of several causative agents of respiratory diseases in PTBS. Thus, this study was intended primarily to assess prevalence of *Cryptosporidium* parasite in the sputa of PTBS at JUMC, Southwest Ethiopia.

1.3. Significance of the Study

Studies have drawn attention to the global burden of cryptosporidiosis which is increasingly identified as an important cause of morbidity and mortality worldwide. Surveillance data about RCI are few or absent in most of African countries. Ethiopia as one of the African developing countries shares the burden of cryptosporidiosis.

Despite the presence of different bacterial, fungal, protozoan and viral respiratory diseases, limited information is available on RCI. This study attempted to provide current preliminary information on the study populations and put baseline figure of RCI for future large scale researches. Moreover, this study will alert microbiology laboratories to suspect the possibility of *Cryptosporidium* oocysts in sputa of PTBS providing as etiological agent of respiratory disorders especially in HIV/AIDS-patients. In turn, early identification of *Cryptosporidium* oocytes can help JUMC for appropriate therapy and reduction of morbidity and mortality in these patients.

2. LITERATURES REVIEW

2.1. Perspective of Respiratory *Cryptosporidium* Infection

Despite of its expected wide distribution and obvious significance to public health, *Cryptosporidium* remains a little-studied protozoan parasite in Ethiopia. It is better known as an intestinal parasitic disease both in immunocompetent and immunocompromised persons of all age groups and both sexes in many countries as well as in Ethiopia. Although the dissemination of chronic IC into respiratory tract is well understood, the global burden of RCI is still under ascertained [6, 31, 49, 50, 51].

2.2. Case Reports of Respiratory *Cryptosporidium* Infection

Since the first report of RCI from tracheal autopsy of a British boy in 1980, a number of cases of RCI were reported at different times across the globe. Twelve cases were reported from different states of United States of America at different period of times [52, 53, 54, 55, 56, 57], 2 cases from Denmark [58, 59], 9 cases from France [7, 15], 2 cases from Italy [60, 61], and from Spain [62, 63], one case from United Kingdom [64], the former Czechoslovakia [5], Chile [4], Iran [65], and Japan [66].

The above scenarios confirmed that almost all cases were from HIV/AIDS-patients which diverted RCI-studies almost solely to these patients. Actually certain cases were also reported from HIV-sero-negative immunocompetent persons from different parts of the world like in Papua, New Guinea, Spain, and India [16, 17, 67, 68].

Even though, we didn't find documented data about RCI in Ethiopia, It has been also identified in immune-competent individuals from distinct African countries. For instance, *Cryptosporidium* oocysts were detected in sputum of 52-years-old Rwandese healthy adult and in the sputum of an 84-year-old South African patient presenting with a persistent productive cough. In addition, most recently RCI studies were also reported from Morocco and Uganda among immuno competent individuals [6, 26, 69]

2.3. Epidemiological Studies of Respiratory *Cryptosporidium* Infection

Generally speaking, little is known about the epidemiology of RCI all over the world specifically in African countries, although a recent review of *Cryptosporidium* in Africa focused on the epidemiology and transmission dynamics. Most of the data are confined to case reports and case series. There are only few epidemiological data about RCI all over the world. Moreover, these studies also engaged in specific study groups like HIV-sero-positive patients, children, immuno depressed person or person with already confirmed IC. Due to specific group of studies, asymptomatic cryptosporidial infections which contribute to the silent dissemination are underappreciated[36, 70].

The few conducted epidemiologic studies demonstrated that *Cryptosporidium* is more prevalent in developing countries (5% to 10%) than in developed countries (1% to 3%) [36, 71, 72, 73]. A survey study conducted in Brazil showed RCI prevalence of 1.5% [74], another prospective study conducted in Spain on 43 AIDS patients with confirmed IC, showed 16.28%[62]. Another study conducted in Uganda among HIV-sero-negative children of age less than 3-years with confirmed IC, revealed 35.4%. and one study has reported a prevalence of 17% in HIV-sero-positive patients with respiratory symptoms[6, 58].

2.4. Factors Contributing for Prevalence of Respiratory *Cryptosporidium* Infection

Susceptibility of human to RCI depends on several factors, including environmental conditions, host immune status, age (children are the most susceptible), geographic location and close proximity or contact with infected humans or animals. Several previous studies on IC but a few on RCI, considered the possible association between prevalence of *Cryptosporidium* within humans and its contributing factors. This study also attempted to assess the variables whether there was or no significant statistical association between different factors and RCI. The available literatures on the prevalence of *Cryptosporidium* with associated risk factors described different risk factors which were mostly categorized under socio-demographic, economic and underlining factors[6, 26, 31].

2.4.1. Socio-demographic and Economic Factors

Human populations all over the world have particular or common factors that make them heterogenic from the other group of population in different aspects of life. Few studies in different parts of the world tried to assess factors that have association with the prevalence of RCI. Different socio-demographic and economic factors (including age, residence, occupation, educational status, monthly income, close proximity to domestic animals) to the occurrence and prevalence of RCI come up with different findings/conclusions [6, 36, 75, 76]

2.4.2. Underlining Factors

The most prominent associated factors that debilitate RCI bearing ability of patients in previous studies were confirmed. Including age, HIV-infection, TB-infection, occupational kind, close proximity to infected animals, chronic IC, and other immunity debilitating factors were described as the most important factors for the prevalence of cryptosporidiosis [4, 6, 75, 77].

3. OBJECTIVES OF THE STUDY

3.1. General Objective

- The general objective of this study was to assess the prevalence of *Cryptosporidium* parasite in sputa of PTBS at Jimma University Medical Center, 2017.

3.2. Specific Objectives

- To assess the prevalence of *Cryptosporidium* parasite in sputa of PTBS
- To identify associated risk factors for the development of RCI in PTBS

4. MATERIALS AND METHODS

4.1. Study Setting

The study was conducted at JUMC, which is found in Jimma Town, Southwest Ethiopia. Jimma Town is located about 352 kilo meters southwest of Addis Ababa, the capital city of Ethiopia. Jimma Town has 3 sub towns and 17 kebeles (the smallest administrative units). It is located at an altitude of 1750 to 2000 m above sea level. The average annual temperature and rainfall range from 20^0 C to 30 ^{0}C, and 800 to 2500 mm^3 respectively. According to the 2007 population and housing census conducted, Jimma Zone has a total population of 2,486,155 with 1,250,527 men and 1,235,628 women within an area of 15,568.58 Km^2, and a total of 521,506 household. The total population of Jimma Town in 2010 was 132,051 (66,343 men and 65,708 women). Jimma Town has two governmental hospitals and three health centers. Jimma University Medical Center which was established in 1938 is now a 640 bedded teaching and referral specialized hospital providing services for approximately 15,988 inpatient, 217,923 outpatient attendants, 13,000 emergency cases and 4500 deliveries in a year coming to it from the catchment population of about 15 million people. Currently it has 1481 staffs (938 technical staffs and 543 supportive staffs)[78].

4.2. Study Period

This study was conducted starting from February 1st to May 5th, 2017.

4.3. Study Design

Hospital-based cross-sectional study was designed and employed.

4.4. Source Population

All clinically suspected PTBS who had been attending JUMC for acid fast bacillus (AFB) smear microscopy were source populations.

4.5. Study Population

All clinically PTBS attending JUMC for AFB smear microscopy during the study period were intended as study populations.

4.6. Inclusion and Exclusion Criteria

4.6.1. Inclusion Criteria

All PTBS of all age and both sex with cough at least for two weeks, and willing to participate in the study by providing written consent and/or assent were eligible.

4.6.2. Exclusion Criterion

Pulmonary tuberculosis suspects who were unable to produce adequate sputum were excluded from this study.

4.7. Sample Size Determination

The sample size (n) was calculated using single population proportion statistical formula of sample size calculation, $n=(Z\alpha/2)^2 P (1-P)/d^2$ [79] .

Where, 'n' and 'Z' stand for the minimum required sample size, and standard score corresponds to 1.96 at 95% confidence interval respectively, '**P'** stands for ideal prevalence of RCI. Since the overall prevalence of RCI around this study area was unknown, a prevalence of 50% was supposed as prevalence of RCI. Whereas'd' is the degree of precision or tolerable error with the assumptions of 95% confidence interval, margin of error (d = 5%). Putting the respective values in the given formula, the final sample size was 385 but considering a 10% non-response rate final sample size of 424 was determined.

4.8. Sampling Technique

Consecutive individuals visiting JUMC for AFB smear microscopy and meeting the criteria of inclusion were enrolled until the required sample size was achieved.

4.9. Study Variables

4.9.1. Dependent Variable

- Respiratory *Cryptosporidium* infection

4.9.2. Independent Variables

- Age
- Average monthly income
- Close proximity to animals
- Difficulty in breathing
- Duration of cough
- Educational status
- Episode of diarrhea
- HIV-infection
- Marital status
- Occupation
- PTB-infection
- Residence
- Sex
- Weight loss

4.10. Operational Definitions

Diarrhea, also spelled diarrhoea: is the condition of having at least three abnormally loose or liquid bowel movements each day[80].

Close proximity to animals: is the state of being nearness, closeness to pet animals with redundancy in contact.

Pulmonary tuberculosis suspect: an individual with a history of cough for two weeks or more, chest pain and difficulty of breathing, unintentional weight loss of body weight, or symptoms of fever for two weeks[81]

Productive cough: cough appeared with expectoration of material from the bronchi of the patients when it lasts longer than eight weeks referred as chronic cough [81]

Tuberculosis: An infectious disease caused by *Mycobacterium* tuberculosis and usually affects the lungs[82].

Weight loss: an unintentional reduction of the total body mass due to a mean loss of fluid, body fat or adipose tissue.

4.11. Data Collection Procedures

4.11.1. Patient Identification, Socio-demographic, Economic and Clinical Data

The patient identification, socio-demographic and underlining conditions assumed to be related with the outcome variables of interest were collected using pretested structured questionnaires after obtaining written informed consent and/or assent from the study participants. The questionnaires were developed in English then translated into Affan Oromo, or Amharic based on the participants linguistic ability then back translated into English. Pre-tested questionnaires were used to collect participants' socio-demographic data and clinical information. Participants or caretakers who were unable to read and write provided their consent or assents with a thumbprint after verbal discussion of the consent document. Selected data like age, educational status, occupation, monthly income, close proximity with animals and their dung, HIV status,

duration of cough, episodes of diarrhea were included. After clear instruction was given to all PTBS by laboratory technologists on how to provide appropriate sputum, about 10 milliliters of sputum samples were collected using dry, clean, leak proof translucent and screw-caped labeled plastic containers at Mycobacteriology Research Center.

4.12. Laboratory Analysis

4.12.1. Smear Preparation

Two spot-spot sputum samples were directly by principal investigator smeared onto labeled glass microscope slides and prepared just like that for Ziehl-Neelsen technique inside label-2 safety cabinets. Moderately thick to thin smears were made with the aid of a wooden applicator stick within an area of about 3 x 1 centimeters. Air drying smears were left in safety cabinets for 30 minutes.

4.12.2. Staining Techniques and Microscopy

Air-dried smears were fixed with absolute methanol inside level-2 safety cabinet for 5 minutes. At staining area, the sputum-smeared and fixed slides were placed in multislide carriers in batches for AP staining according to standard operational procedure (SOP) (Annex-1). Then the AP-stained smears were initially scanned under 20x and 40x magnifications of Primo Star (ZEISS 415500-Germany) light emitting diode (iLED) fluorescent microscope to detect typical bright fluorescence *Cryptosporidium* oocysts against dark background, at least 50 fields were examined for each slide. When fluorescing oocysts were observed with appropriate morphology and size within AP-stained slides, IFAT (aqua-Glu^{TM} kit New Orleans, USA, with production & expired date 3/2016 and 12/2018- respectively) staining technique was applied on the same previously AP-stained slides within a day, and examined under 20x and 40x magnifications of Primo Star iLED fluorescent microscope with similar fashion to screening of AP-stained slides. When fluorescing oocysts were also detected during IFAT examination, the smears were confirmed as positive for *Cryptosporidium* oocysts. Both AP and IFAT staining techniques were taken place within a day of sample reception. For this study, IFAT was intended as definitive identification and confirmation tests.

4.12.3. Wet Mount Preparation in Potassium Hydroxide

Wet mount preparations in 10% potassium hydroxide (KOH) were made on all *Cryptosporidium* oocysts positive sputa. The mounted slides were examined under light microscope using the 10X and 40X objective lenses to look for the presence of fungi and fungal elements.

4. 12.4. Sputum Cultures

Study participants, whose sputum specimens were positive for *Cryptosporidium* oocysts in AP and IFAT examinations, were requested to resubmit sputum with sterile falcon tubes, and then these positive sputum samples were inoculated onto chocolate agar and sheep blood agar plates.

4.13. Data Management and Statistical Analysis

The checked and coded data were entered into Epi-data version 3.1. After cleaning for incompleteness, inconsistencies and missing values, edited data were exported to SPSS version 21 computer software. Descriptive statistics using frequencies, percentages, mean, and standard deviations were used to describe socio-demographic characteristics and the findings. Cross-tabulation between each explanatory variable and the outcome variable were conducted using chi-square or Fisher exact test. Based on the fulfillments of the assumptions of chi-square or Fisher exact test, all explanatory variables which have association with the outcome variables was determined as the presence of statistically significant associations between explanatory variables and the outcome variable at P value < 0.05.

4.14. Quality Assurance

Short term training about the objective of study and process of data collection was given for data collectors by principal investigator for two days. After collection, data were checked for their completeness at the end of each data collecting day.

Standard operating procedure was properly employed for every laboratory procedure (Annex II). The reliability of the study findings were guaranteed by conducting AP and IFAT quality controls every day before each step of laboratory tests in the entire process. Also the accurate performance of the microscopy was assured with both negative and positive stained slides. All slides were seen by principal investigator and positive slides were rechecked by three senior laboratory professionals. The culture media were prepared according to the procedures of manufacture instruction and SOP. Media sterility was checked by incubating randomly designated plates of each type of media plates. For all quality activities the necessary modifications were done based on the findings.

4.15. Ethical Consideration

After the proposal of this study was reviewed and approved by Ethical Review Committee of Institute of Health, Jimma University. Ethical clearance and support letter was received and submitted to JUMC. Objective of the study was further explained in addition to the letter to JUMC officials to obtain permission. Being informed about purpose of the study, all study participants had given verbal and written informed consent before enrollment into the study to confirm their willingness to participate in the study. When the study participant was a child, age categories less than 18-year-old, an assent from the child and additional written consent from his or her parent or guardian was obtained. Those can't read and write provided their written consent and/or accent with finger print. As far as privacy and confidentiality was concerned, the collected data were kept with a firm confidentially. For those participants whose laboratory results were positive, the principal investigator reported and consulted to the concerned physician for further management.

4.16. Dissemination of the Study Results

The finding of this study was submitted to School of Medical Laboratory Sciences, Institute of Health, Jimma University as part of Master of Science thesis and will be presented to wider audience during public defense. Summary of the study will be communicated to different stakeholders of JUMC as report. Again, the study finding will be presented in different work shop and scientific seminars as much as possible. Finally the paper will be submitted to peer-reviewed journals for publication in either national or international peer-reviewed journal.

4.17. Data Back up

The entire data and final finding of this study are kept both in hard copies and soft copies such as in flash-memory, compact disk and emailed online for secured uses.

5. RESULTS

5.1. Socio-demographic and Economic Variables of Pulmonary Tuberculosis Suspects at Jimma University Medical Center, 2017

A total of 424 PTBS were recruited in this study. The overall mean age of the participants was 39.3 years (± 16.6 SD) ranging from 6 to 82 years. Out of these, more than half, 234 (55.2%) were male and 190 (44.8%) were female with male to female ratio of 2.6:1. The frequently seen age groups both in males and females were 26-35 years (25.7%) (**Table 1**).

Table 1: Socio-demographic and economic variables of pulmonary tuberculosis suspects at Jimma University Medical Center from February 1st to May 5th 2017

Variables	Category	Frequency (%)	Variables	Category	Frequency (%)
Age in year	5-15	16(3.8)	Occupation	Farmer	131(39.9)
	16-25	83(19.6)		Laborer	48(11.3)
	26-35	109(25.7)		House wife	55(13.0)
	36-45	83(19.6)		Student	52(12.3)
	46-55	45(10.6)		Govt/NGO employee	58(13.7)
	56-65	57(13.4)		Merchant	51(12.0)
	>66	31(7.3)		Others *	29(6.8)
Residence	Rural	240(56.6)	Marital status	Single	109(25.7)
	Urban	184(43.4)		Married	267(63.0)
Educational status	Illiterate	49(11.6)		widow	11(2.6)
	Can read & write	104(24.5)		Divorced	19(4.5)
				Others **	18(4.2)
	Grade 1-4	74(17.5)	Family monthly average income	≤ 500	70(16.8)
	Grade 5-8	66(15.6)		501-1000	96(22.6)
	Grade 9-12	60(14.2)		1001-1500	98(23.1)
	College & above	71(16.7)		1501-2000	66(15.6)
				2001-3000	36(8.5)
				3000-4000	26(6.1)
				4000-5000	17(4.0)
				>5000	15(3.5)

Note * = drivers, cattle –keepers. ** Below 18-years-olds participants

5.2. Clinical Characteristics of Pulmonary Tuberculosis Suspects whose sputa were tested for *Cryptosporidium* oocysts at Jimma University Medical Center, 2017

The indicative clinical characteristics of RCI were observed in most study participants, particularly in *Cryptosporidium* oocysts positive participants. Productive cough was extremely prevalent and evident in all 424 (100%) study participants, as self-reported with different duration; 50 (11.8%) had cough from 2 to 4 weeks, 170 (40%) had cough from 4 to 6 weeks, and 204 (48.1%) had cough more than 6 weeks.

On the other hands, only 43 (10.1%) and 60 (14.2%) of study participants had complained prolonged diarrhea and weight loss respectively during the last two weeks before this assessment was done. Difficulty in breathing, as self-reported during coughing, was complained among 391 (92.2%) of 424 study participants.

Pulmonary TB-infection (i.e. the presence of acid-fast-bacilli) was detected in 9 (2.12%) study participants of 424 PTBS, of these, three cases were exclusively detected by AP-staining technique during scanning of *Cryptosporidium* oocysts while the rest six cases were detected both by AP & Ziehl-Neelsen staining techniques. *Cryptosporidium* oocysts were detected as co-infection among three of these nine PTB patients. Similarly *Cryptosporidium* oocysts were detected as co-infection among three of ten PTBS with HIV-infection. One study participant had both *Cryptosporidium* oocysts and acid-fast bacilli in his sputum. He also complained of prolonged watery diarrhea (**Table 2**).

Most of study participants 300 (70.75%) had close proximity to ether livestock, hens or pet animals in their day to day activities; among all 240 rural dwellers, 202 (84.17%) and among from 184 urban dwellers 98 (53.26%) had close proximity to animals and continuous exposure to their dung. Mainly almost all farmer participants 122/131 (93.1%) from rural areas had continuous direct contact with their livestock animals.

Table 2: Clinical characteristics of pulmonary tuberculosis suspects whose sputa were tested for *Cryptosporidium* oocysts at Jimma University Medical Center, from February 1st to May 5th 2017

Variables	Category	Frequency	Percent (%)
Cough	Yes	424	100.0
	No	0	0.0
Duration of cough	2-4 weeks	50	11.8
	4-6weeks	170	40.1
	>6 weeks	204	48.1
Weight loss	Yes	46	10.8
	No	378	89.2
Difficulty in breathing	Yes	391	92.2
	No	33	7.8
HIV status	Positive	10	2.4
	Negative	414	97.6
PTB	Positive	9	2.1
	Negative	415	97.9
Diarrhoea	Yes	43	10.1
	No	381	89.9

5.3. Socio-demographic and Economic Variables of *Cryptosporidium* Positive Pulmonary Tuberculosis Suspects at Jimma University Medical Center, 2017

Cryptosporidium oocysts were detected in the sputa of 9 participants among 424 PTBS participated in the study with overall prevalence 2.12%. Five of nine positive sputum samples (four patients were missed to resubmit morning sputum specimens) were inoculated onto sheep blood agar and chocolate agar plates, and wet mount preparations of KOH were made to assess the presence of bacteria and fungus and/or fungal elements respectively, but significant respiratory pathogens were neither grown in culture media nor observed in KOH wet mount preparation. The overall mean age of the *Cryptosporidium* positive PTBS were 35.8-years (SD± 28.1 years) ranging from 7 to 75-years. More than one-third of *Cryptosporidium* positive study participants were in age groups of 5-15 years and more than 66 years (**Table 3**).

Table 3: Socio-demographic and economic variables of *Cryptosporidium* positive pulmonary tuberculosis suspects at Jimma University Medical Center, from February 1st to May 5th, 2017.

Variables	Category	Frequency (%)	Variables	Category	Frequency (%)
Age	5-15	3(33.3)	Occupation	Farmer	4(44.4)
	16-25	1(11.1)		Laborer	0(0.0)
	26-35	1(11.1)		House wife	0(0.0)
	36-45	0(0.0)		Student	0(0.0)
	46-55	0(0.0)		Govt/NGO employee	1(11.1)
	56-65	1(11.1)		Merchant	0(0.0)
	>66	3(33.)		Others *	4(44.4)

				Single	2(22.2)
				Married	4(44.4)
Sex	Male	6(66.7)	Marital status	widow	0(0.0)
	Female	3(33.3)		Divorced	0(0.0)
				Others **	3(33.3)
				≤ 500	1(11.1)
				501-1000	2(22.2)
			Family monthly average income	1001-1500	2(22.2)
Residence	Rural	7(77.8)		1501-2000	2(22.2)
	Urban	2(22.2)		2001-3000	2(22.2)
				3000-4000	0(0.0)
				4000-5000	0(0.0)
				>5000	0(0.0)
	Illiterate	2(22.2)			
	Can read & write	0(0.0)			
Educational status	Grade 1-4	4(44.4)	Close proximity to animals	Yes	9(100)
	Grade 5-8	3(33.3)		No	0(0.0)
	Grade 9-12	0(0.0)			
	College & above	0(0.0)			

Note * = drivers, cattle –keepers. ** Below 18-years-olds participants

5.4. Clinical Characteristics of *Cryptosporidium* Positive Pulmonary Tuberculosis Suspects at Jimma University Medical Center, 2017

Eight of nine of the *Cryptosporidium* positive PTBS, had chronic cough for more than 6 weeks, while only one person had productive cough between 2 to 4 weeks before they came to JUMC for AFB diagnosis. All the nine *Cryptosporidium* positive individuals were tested for HIV-infection at a time when oocysts were found in their sputa, 3 (30.0%) of these *Cryptosporidium* positive 9 PTBS had HIV-infection. One woman, who didn't yet initiate antiretroviral therapy, had PTB-infection. As self-reported, she was treated with anti-TB treatment regimes, and she had been cured, but still with productive cough. Another *Cryptosporidium* positive man had been co-infected with PTB and HIV. Prolonged diarrhea was complained in 9-years-old and 11-year-old sister kids. These female cattle-kippers sister kids were admitted at JUMC for a week complaining prolonged diarrhea and chronic cough. Both these cattle-keepers kids had close proximity to their cattle.

Table 4: Clinical characteristics of *Cryptosporidium* positive pulmonary tuberculosis suspects at Jimma University Medical Center, from February 1st to May 5th, 2017

No	age	Sex	Duration of cough (in week)	Difficulty in breath	Weight loss	PTB infection	HIV infection	diarrhea
1	7	M	>6	Yes	No	No	No	Yes
2	9	F	>6	Yes	No	No	No	Yes
3	11	F	>6	Yes	Yes	No	No	Yes
4	20	M	2-4	Yes	No	No	Yes	No
5	26	M	>6	Yes	Yes	Yes	Yes	Yes
6	35	F	>6	Yes	Yes	No	Yes	No
7	72	M	>6	Yes	Yes	Yes	No	No
8	67	M	>6	Yes	Yes	No	No	No
9	75	M	>6	Yes	Yes	Yes	No	No

5.5. Risk Factors Associated with Respiratory *Cryptosporidium* Infection

The possible different risk factors within socio-demographic variables including age, residence, average monthly family income, close proximity to animals, educational status, marital status occupational kind and sex, or underlining clinical factors such as episode of diarrhea, difficulty in breathing, weight loss, HIV-infection and PTB-infection which might have association with prevalence of RCI were assessed whether they had associations or not. But most of these variable were not statistically significant factors for the prevalence of RCI (P>0.05) (**Table 5**).

Table 5: Statistical tests of variables insignificantly associated with respiratory *Cryptosporidium* infection in pulmonary tuberculosis suspects at Jimma University Medical Center, from February 1st to May 5th, 2017

Variables	RCI/total No(%)	X^2 statistics	
		value	p-value
Age (year)		0.36	0.74
5-15	3/16 (18.7)		
16-25	1/83 (1.2)		
26-35	1/109 (0.9)		
36-45	0/83(0.0)		
46-55	0/45(0.0)		
56-65	1/57 (1.8)		
>66	3/31 (9.7)		
Sex		0.49	0.48
Male	6/228 (2.6)		
Female	3/187 (1.6)		
Educational status			

Variables	RCI/total No (%)	X^2 statistics	
		value	p-value
Marital status		3.73	0.86
Single	2/107(1.9)		
Married	4/263(2.5)		
widow	0/11(0.0)		
Divorced	0/18(0.0)		
Others **	3/16 (18.8)		
Weight loss		8.73	0.99
Yes	1/38(2.6)		
No	8/386(2.1)		
monthly income (birr)		3.7	0.81
	1/69 (1.4)		

Illiterate	2/47 (4.3)			≤ 500	2/94 (2.1)		
Can read write	0/104(0.0)				2/96 (2.1)		
Grade 1-4	4/70 (5.7)			501-1000	2/64 (3.10		
Grade 5-8	3/63 (4.8)			1001-1500	2/34 (5.9)		
Grade 9-12	0/60(0.0)				0/26(0.0)		
College& above	0/71(0.0)			1501-2000	0/27(0.0)		
				2001-3000	0/15(0.0)		
				3000-4000			
				4000-5000			
				>5000			
Residency		1.69	0.19	**Episodes of diarrhea**		5.4	0.053++
Rural	7/233 (3.0)						
Urban	2/182 (1.1)			Yes	3/40 (7.5)		
				No	6/384(1.6)		

Note ++ = Fisher's Exact Test, ** below 18-years-olds participants

On the other hands, statistically significant risk factors for the prevalence of RCI in this study were close proximity to animals (P value = 0.049), HIV-infection (P-value= 0.001), occupational kind (P- value = 0.04) and PTB-infection (P value =0.001) (**Table 6**).

Table 6: Statistical tests of variables that showed significant association with respiratory *Cryptosporidium* infection in pulmonary tuberculosis suspects at Jimma University Medical Center, from February 1st to May 5th, 2017**.**

Variables	**RCI/total N_O (%)**	**X^2 statistics**	
		value	p-value
Occupation		24.07	0.04++
Farmer	4/127 (3.1)		
Laborer	0/48 (0.0)		
House wife	0/55 (0.0)		
Student	0/52 (0.0)		
Govt/NGO employee	1/57 (1.8)		
Merchant	0/51 (0.0)		
Others *	4/25 (16.0)		
Close proximity to animals		3.8	0.049
Yes	9/291 (3.1)		
No	0/133 (0.0)		
HIV infection		34.38	0.001
Positive	3/10 (30.0)		
Negative	6/414 (1.4)		
PTB infection		43.11	0.001
Positive	3/9 (33.3)		
Negative	6/415 (1.4)		

Note ++ = Fisher's Exact Test, *= drivers, cattle –keepers

6. DISCUSSION

Respiratory *Cryptosporidium* infection is currently recognized as a rare, late-stage complication of chronic IC especially in persons with HIV/AIDS infection. Since the early 1980, to the most recent reviews of RCI all over the world, several cases were reported in persons with AIDS, while about few cases documented in persons with immunodeficiency due to other causes including malnutrition, solid organ-transplantation and corticosteroid-therapy. But recent reports revealed that RCI also occurred in immune competent persons. Still there have been few studies on RCI, as these early studies focused either on adult with HIV/AIDS or on children with confirmed IC [6, 47, 63].

From a total of 424 PTBS took part in this study, we found that 9 (2.12%) had *Cryptosporidium* oocysts in their sputa. Although the diagnostic methods and study populations varied each other, the present prevalence of RCI in this study is in agreement with previous studies conducted in Spain and Brazil that showed prevalence of 2.02% and 1.5% respectively. [63, 76]. Those studies were conducted among HIV sero-positive and HIV sero-negative individuals by using Ziehl-Neelsen staining techniques. On the other hands, in contrast to our findings, the prevalence of RCI was found somewhat different in previous studies conducted in Uganda among PTB-infected adults 3.6 % [83], and in Indonesia among PTB-infected patients 6.8% [84]. Again another study conducted in Uganda, showed much higher prevalence 34.5% [6] among under 3-year-old children with confirmed IC.

These differences might be related due to variation in diagnostic methods, study population, immunity status, or underlining features in the study populations. Both those studies conducted in Indonesia and in Uganda, the former one, employed modified version acid fast stain of Ziehl Neelsen technique. While the latter study conducted in Uganda used polymerase chain reaction (PCR) technique which has extreme sensitivity and specificity. Additionally the diagnostic methods were intended to detect *Cryptosporidium's* DNA in children's induced sputa rather than detecting directly the *Cryptosporidium* oocysts which enhance the detecting rate of the parasite. Unfortunately, no comparable documented results elsewhere because most of reports of RCI were either from case reports or from case serious studies.

In our study, all the nine positive sputa for *Cryptosporidium* oocysts by the AP-staining technique were confirmed with IFAT staining as positives. Among several reasons, one possible explanation for such low prevalence of RCI in this study might be due to AP-staining as screening test which has moderate sensitivity in comparison with PCR with superior sensitivity and specificity and IFAT (the aqua-Glo^{TM} kit sensitivity 98.5% and specificity 100%) staining technique. Because of its expensive cost IFAT was employed only to the nine sputum specimens (*Cryptosporidium* oocysts positive by AP-staining screening) as confirmatory test, despite the fact that prior studies shown that immunological methods of detecting *Cryptosporidium* antigen in sputum samples as rapid tests are becoming more popular and they have an advantage over the modified acid-fast staining technique.

This study also attempted to assess the intended variables weather they had statically significant association or not for the development of RCI at 95% confidence interval. Most of these parameters from the socio-demographic variables of study participants, age, average family monthly income, educational status, marital status, residence and sex were found not to have statistically significant association with RCI. Again from the underlining and clinical factors, difficulty in breathing, duration of cough, episode of diarrhea and weight loss had no statistically significant association with the prevalence of RCI, in PTBS ($P>0.05$).

Unlike this present study, age, episodes of diarrhea, rural residence, were significantly associated with prevalence of RCI according to previous studies in Uganda[6]. Residence either in rural or urban setting was another significant factor related with development of RCI, which should be taken into consideration. Rural dwellers are found to more prone to develop RCI. This significant association may be due to rural dwellers having more frequent contacts with their livestock animals which may have already been infected with cryptosporidiosis. These difference may be due difference in the socio-demographic characteristics and life style of study population. The reason for this finding may be also due to few number of cases (9-cases) and small sample size of our study in comparison of previous studies.

From the assessed underlining conditions and clinical features in our study, close proximity to animals, occupation-kind, HIV-infection and PTB-infection had statistically significant associations with the prevalence of RCI. These findings were in agreement with the previous

studies from Uganda[83]. Close proximity to animals seems to be a significant source of zoonotic transmission, mainly in rural areas. This may occur from household pets, livestock, sheep or goats. *Cryptosporidium* infection causes more economic losses to animal husbandry and livestock production ,in Holeta Town, central Ethiopia 40% of dairy cattle calves have been found to be infected with cryptosporidiosis and these animals may also be assumed to serve as reservoir of infection for humans[41].

The present findings also revealed that prevalence of RCI in rural areas was higher than those who live in urbanites areas. This could probably be due to close proximity to livestock animals, poor environmental sanitation and personal hygiene which have high risk for the development of IC and indirectly to RCI [85].

However single infections with *Cryptosporidium* parasite have also been observed, RCI frequently results from co-infection with other pathogens, like *Mycobacterium tuberculosis* or *Pneumocystis carinii* [13, 63]. Even though, we also found three cases of RCI patients were co-infected with PTB, it was unable to identify the cause of the diseases. Again other three cases of RCI were found as co-infection with HIV-infection. This may be due to immunosuppression by PTB-infection and HIV-infection. Both PTB-infection and HIV-infection had statistically significant association in the prevalence of RCI. This might be because HIV/AIDS is well known to undermine the natural immunity systems. Similarly, PTB is a chronic respiratory disease and tending to cause a weakened immune system (immunocompromised).These associations of PTB-infection and HIV-infection with prevalence of RCI were in agreement with previous studies conducted in Morocco and Spain[26, 63]. In addition it should be noticed that the previous case reports and case serous of RCI were almost from those immunocompromised patients either by HIV/AIDS or other perspective diseases.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

In conclusion, although the prevalence of RCI in PTBS in this study area looked like low, it indicated the importance of RCI as a public health problem. Particularly, we believe the present finding presents RCI to the attention for researchers and physicians to consider the possibility of ruling out RCI in PTBS. This study also showed close proximity to animals, occupation kind, infections with HIV and TB were factors significantly associated with RCI in the studied population.

7.2. Recommendations

Based on the finding of this study the following recommendations are forwarded.

- Considering the possibility of RCI in PTBS, it is better if medical laboratory personals, who conduct routine AP microscopy for PTB bacilli, should be familiar with the appearance of *Cryptosporidium* oocysts in respiratory specimens.
- It is better to conduct large scale studies with stronger design to provide updated information for further understanding of RCI to appreciate the full spectrum.
- Providing health education for the community about mode of transmission is needed to prevent RCI.

8. STRENGTHS AND LIMITATIONS OF THE STUDY

8.1. Strength of the Study

Since it is the first study across the country, it can figure out the magnitude of the problem in the study groups. Similarly the present finding of this study, prevalence of RCI and its association risk factors are better approaches for future researches.

8.2. Limitations of the Study

The low sample size used in this study could either underestimate or overestimate the results. Since this study was hospital based, the assessed prevalence may not reflect the actual situation of RCI in asymptomatic carriers in the community.

Using microscopic techniques rather than PCR, may decrease the detecting rate of *Cryptosporidium* oocysts in sputum. Thus if PCR and IFAT were used for all samples, the prevalence might be higher than what has been reported in this study.

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ANNEXES

Annex-I. Laboratory Producers

A. Auramine Phenol-Staining Technique

Sputum Smear Fluorescence Microscopy

Cryptosporidium oocysts retain the primary stain, the auramine phenol solution even after exposure to decolorizing with acid alcohol. A counter stain is employed to highlight the stained organisms for easier recognition. Potassium permanganate is used as counter-stain and it helps prevent non-specific fluorescence.

Required Reagents:

1. Auramine phenol solution----------0.1%
2. Acid alcohol----------0.5%
3. Potassium permanganate----------0.1%
4. Absolute methanol----------100%

Storage of Reagents: The stock solution should be stored at 2 to 25°C temperature in dark place. Don't exposure to direct light.

Procedures

1. Select a new, unscratched slide and label it with a laboratory serial number.
2. As quickly as sputum specimen submitted to JUMRC, make smear onto glass slides (medium to thick) in side lebel-2 bio safety cabinet with wooden applicator stick within an area of about 3 cm x 1 centimeter.
3. Let the smear air-dry for 30 minutes in side lebel-2 bio safety cabinet.
4. Fix the dried smear with absolute methanol for 5 minutes then allow air dry.
5. Place slides on a staining rack, with the smeared side facing up, not touching each other.
6. Flood the slides with freshly filtered 0.1% auramine phenol. Let stand for 20 minutes.
7. Wash well with tap water, taking care not to wash away the smear.

8. Decolorize by covering completely with 0.5% acid-alcohol (HCl-methanol) for 3 minutes
9. Wash the slide well with tap water, as before to wash away the acid alcohol.
10. Counter stain with 0.1% potassium permanganate for 2 minutes.
11. Wash with tap water and slope the slides on slid tracks to air dry for 30 minutes.
12. Examine with 20x and objective with 10x eyepiece lens of Primo Star iLED FM. At least, a minimum of 50 fields should be examined.

Interpretations:

Positive Result: Cryptosporidium oocysts (4-6μm diameter) are ring or doughnut-shaped and fluoresce greeny-yellow against a dark field background. It is supposed that oocysts may be measured by increasing the bright field light intensity and measuring the oocysts with a calibrated eye-piece micrometer.

Negative Result: If no fluorescent objects are seen against a dark field background with the appropriate shape and size for Cryptosporidium oocysts, then tests are considered as negative results

B. Immuno Fluorescent Anti body Test- Staining Technique

The IFAT is monoclonal antibody against Cryptosporidium oocysts antigens with excellent sensitivity and specificity. For this particular study, the aqua-Glo™ kit is designed to detect *Cryptosporidium* oocyst antigens in particular isolated forms of clinical samples (sputum) utilizing the principle of direct fluorescence. The antibody reagent consists of a mixture of fluorescein-labeled mice monoclonal antibodies made to oocysts outer wall antigenic sites, the epitopes of *Cryptosporidium* spp. This reagent is genus-specific and will bind only to oocysts of Cryptosporidium. The oocysts will appear bright apple green when viewed under fluorescent microscope.

Required Reagents:

1. Aqua-Glo antibodies
2. Counter stain
3. sureRinse buffer solution

Storage of Reagents: Store at 2 to 4 ^{o}c. Don't freeze and keep in dark place.

Procedures

1. Add 5-15 µl of Aqua-Glo antibodies onto *Cryptosporidium* oocysts-positive slides which were stained with auramine phenol staining.
2. Incubate sample in humid chamber (box with damp tissue and lid) place at room temperature for 40 minutes or longer.
3. Tap off surplus Mab (by tilting the slide, long edge down, and absorb excess fluid with soft paper placed at the edge of the slide well.)

a. Gently add 1 drop of water to cover well (do not squirt directly on to well).

4. Leave water drop on for 1 minute. Tap off.
5. Apply 1 drop of counter stain per well
6. Incubate for 1 minute at room temperature
7. Rinse the slide free of counter stain by adding 1 drop of water.
8. Place a drop of mounting medium or water before covering with cover slip.
9. Examine with 20x and 40x objective of iLED FM at least of 50 fields.

Interpretations

Positive Result: Cryptosporidium oocysts (4-6µm diameter) are ring-shaped, usually circular, sometimes slightly oval, and fluoresce greeny-yellow against a dark field background. Putative oocysts may be measured by increasing the bright field light intensity and measuring the oocysts with a calibrated eye-piece micrometer**.**

Negative Result: No fluorescing objects with the precise shape or size of oocysts**.**

Quality Control: Include Cryptosporidium positive control and a negative control every time IFAT microscopy is performed, minimum 1 positive and 1 negative well per 9 sample wells and mix the positive and negative control vials before use.

Annex-II Information Sheet

A. English Version

Title of the project: Respiratory Cryptosporidium infection in pulmonary tuberculosis suspects at Jimma University Medical Center, Southwest Ethiopia.

Name of Principal Investigator: Paulos Fissiha

Organization: Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Name of sponsors: Jimma University

This information sheet was prepared for those PTBS attending JUMC during the study period and fulfilled our inclusion criteria intended for the study. I am going to tell you the objective of the study and about the whole processes. I request you to participate in the study voluntarily.

Description and Purpose of the Study: Respiratory Cryptosporidium infection, which is one of neglected diseases but becoming a concern especially among immunocompromised patients, is now emerging as a major problem in the worldwide. Therefore, this study will assess the prevalence of RCI in PTBS. So it will help to put appropriate laboratory diagnostic methods and effective treatments based on the prevalence results of RCI. At large, this study will contribute to health service providers to set regular sputum diagnosis of RCI thus with better diagnosis and treatment in the reduction of morbidity and mortality.

Procedures of Sampling: If you are willing to join the study, you will be requested to sign or stump with thump-print the consent or assent form. Your socio-demographic information, underling symptoms and data of the possible risk factors associated with RCI will be asked. The interview takes a maximum of 10 minutes. Then your sputum given to AFB work up will be processed with AP & IFAT at Mycobacteriology Research Center to detect Cryptosporidium oocysts. If *Cryptosporidium* oocysts are detected you will be asked to resubmit your sputum for culture process.

Risks and Discomforts: Due to participating in this study there will be no risk or discomfort occurred on you at all.

Benefits and Compensation: Being recruited and participating in this study, there will not be direct financial benefit. But if Cryptosporidium oocysts are detected in your sputum, then it will be reported to the physicians who examined you for further care and treatment.

Confidentiality: All information that will be collected from the study participants will be kept confidentially. Any information about the participant will be stored in a file that will not bear a name on it; instead only specified number will be assigned. Unless signed permission of the study participants, the information will not be disclosed to others.

Voluntary Participation and Withdrawal: Your participation is based on your voluntary; primarily, either you may decide not to participate or withdraw the study at any time, your decision will not result in any loss of benefits, not put you at risk in present or future medical care or other benefits to which you otherwise entitled.

If you have any doubt, questions or suggestions about the study, you can ask the study investigators listed below using the following addresses.

Names	Mobile Phone numbers	E-mail addresses
1 Paulos Fissiha (BSc.)	. +251 913 91 45 71	. egullego7@gmail.com
2 Alemseged Abdisa (PhD)	. +251 931 05 71 95	. alemseged.abdissa@gmail.com
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4 Wakjira Kebede (MSc)	. +251 911 37 83 86	. wakjirakebede@yahoo.com
5 Zeleke Mekonnen (PhD)	. +251 917 76 54 27	. zeleke.mekonnen@ju.edu.et
6 Gemeda Abebe (PhD)	. +251 911 99 12 85	. moa.ayana@gmail.com

B. Afan Oromo version

Unkaa Odeeffannoo

Maqaa Projektii:-Respiratory Cryptosporidium infection in pulmonary tuberculosis suspects at Jimma University Medical Center, Southwest Ethiopia

Maqaa Qu'ataa: Paauloos Fissihaa

Dhaabbataa: Yuunivarsitii Jimma, Muummee Barnoota School of Laaboraatorii Saayinsii.

Maqaa Spoonsaraa: Yuunivarsiitii Jimmaa

Unkaan odeeffannoon kuni kan qophaa'e shakkamtoota dhukkuba sombaata'anii warroota qorannoo kana irratti hirmaataniif yommuu ta'u,isaanis walqabatee wantoota qorannoon kun qabatuu ffedhii keessaniin qofa kan hirmaattan ta'uu isaa kabajaan an isini iibsa.

Faayidaa qoranno: dhibeen sombaa dhukkuba yeroo amma addunyaa keenya sodaachisaa jiruu dha.Keessattu biyyoota guddataa jiran kanneen akka Itiyoophiyaa irratti darancimaa jira. Dhibeen kunis afuuraan namarra gara namaatti kantamsa'uu dha. Kanaafuu, qorannoon kunis filannoo addaa shakkamtoota ismiir negativii dhibee sombaatiif ta'a.

- Adeemsa qorannoo
- kaardiin dhukkubsataa ni-ilaallama
- daqiiqa kudhaniif waliin turra
- akkitaa ni kennitu
- Akkitaan fudhatame Yuuniversitii Jimmaatti, Institiyuutii Maayikoobaacteriyoolojitti yommuu hojjamu,qorannoon hojjatamuus:molecular i maayikrooskoppii fi culture tu hojjetama.

Yaaddoo fi miidhaa: Akkitaa kennuu fi yeroo jedhametti hayyamamaa ta'uun yaaddoo fi rakkoo isinirraan ga'u hinjiru.

Faayidaahirmaannaa: Qorannoo kana irratti hirmaachuun keessaniif faayidaan qarshiidhaan argattan jiraachuu baatuus, dhibeen sombaati fi HIV'n isin keessatti yoo argame yaalii dhaaf gara doctariti ni ergamtu.

Iccitii:- odeeffannon qorannoo kana irra argamu hundaafuu iccitiinni eeggama. Odeeffannoon qorannoo kana irra aargamu, maqaa keessan kan hin qabannee fi lakkoofsota qorannoof kennamaniin kaa'amanii kan bakka bu'anta'a.

Fedhii hirmaachuu: Qorannoo kana irratti kan hirmaattan fedhiin qofa yommuu ta'u , yoo hin barbaannee ta'e yeroo barbaaddanitti addakutuu nidandeessu. Filannoon keessan ammas ta'ee fulduraaf maamiltummaa keessanirratti fi faayidaa argattani walitti dhufeenya hinqabu. Qorannoo kana wajjin walqabatee wanta ifa isiniif hin taane ammas ta'ee fulduraaf gaafachuu ni dandeessu.

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milkaa'ina qoranoo kanaaf gaaffilee armaan gaditti tarreeffamaniif deebii sirrii laachuu dhaan akka nu gargaartan kabajaan isin gaafanna.

C. የአማርኛ ግልባጭ

የጥናቱ ርዕስ፡ “ የትንፈሳ ክራይፐቶስፖሪዲዮሲስ ተሃዋስያን በሳንባ ነቀርሳ እንደ ተጠቁ ተጠርጥረው ወደ ጅማ ዩኒቨርሲቲ የሕክምና ማእከል መጥተዉ በሚታከሙ ታካሚዎች ውስጥ ምን ይመስላል” የሚል ነው ።

ዋና ተመራማሪ: ጳውሎስ ፍሥሓ

ድርጅቱ፡-በጅማ ዩኒቨርሲቲ የሕክምና ማእከል ፣ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል ::

የገንዘብ ድጋፍ የሚያደርገዉ ድርጅት፡ - ጅማ ዩኒቨርሲቲ

ይህ የመረጃ ቅጽ የተዘጋጀዉ ከላይ በተጠቀሰው ጥናት ለሚሳተፉ ታካሚዎች ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሂዳቸው ስለ ፈለግናቸው ጉዳዮች እና ስለ ጥናቱ ጠቅላላ ማብራሪያ ለመስጠት የታለመ ነው፡፡ በመሆኑም የጥናቱን መስፈርት አሟልተው የተገኙ ሁሉም ታካሚዎች በጥናቱ የሚሳተፉት በራሳቸዉ ፍላጐት ብቻ መሆኑን በትህትና እንገልፃለን፡፡

ስለ ጥናቱ መግለጫ በጥቂቱ: ክራይፐቶስፖሪዲየም በመባል የሚታወቀው የፕሮዞዋ ተሃዋሲ በሽታ የመከላከል አቅማቸው የተዳከሙ እና ያልተዳከሙ ሰዎች ውስጥ በመናኘት የላይኛውን እና የታችኛውን የመተንፈሻ አካላትን በማጥቃት ከሳንባ ነቀርሳ ጋር ተመሳሳይ የሆነ ምልክት በማምጣት ሰዎችን ያጠቃል :: በሽታው በኢትዮጵያ ጭራሽ በዓለም አቀፍ ደረጃ ደግሞ ብዙም ጥናት ባለመደረጉ እና የህክምና ትኩረት ባለመሰጠቱ ለከፍተኛ የመተንፈሻ በሽታ እና ለሞት አደጋ ብዙዎችን እንደሚዳርግ ይገመታል :: በመሆኑም ይህ ጥናት የክራፕቶስፖሪድዮሲስ በሽታ አምጪ ተሃዋሲ ክፍለ-አካላት በሳንባ ነቀርሳ እንደተጠቁ የተጠርጥሩ ታካሚዎችን ወደ ጅማ ዩኒቨርሲቲ የሕክምና ማእከል ለቲቢ ምርመራ ከሚታከሙ ታካሚዎች ላይ ስርጭቱ ምን እንደሚመስል ለመዳሰስ ይጠቅማል። ከዚህ መተጨማሪ ደግሞ ተዋሐስያኑ የተወሰኑ የጎብረተ ሰብእ ክፍሎችን ለይቶ የሚጠቃበት ዋና ዋና ተዛማጅ ምክንያቶችን ይዳስሳል ። ለታካሚው የሚያበረክተውን ጊዜያዊ አስተዋስኦ ስናይ ታካሚውን ከሃኪሞችም ጋር በወቅቱ በማገናኘት ተገቢህክምና እንዲያገኝ ይደረጋል። በጠጨማሪም በተጠናዉ ጥናት ዉጤት ላይ በመመርኮዝ ትክክለኛ ሕክምና ለታካሚዎች ለመስጠትም ይረዳቸዋል፡፡ የወደፊት ጠቀሜታውን ስንመለከት ደግሞ በበሽታው ዙሪያ ጥናት ለሚያካሂዱ ተመራማሪዎች መሰረታዊ የክራይፐቶስፖሪዲየም መረጃ ሆኖ የሚያገለግል ሲሆን በጤና ተቋማት ዉስጥ እቅድ ለሚያዘጋጁት አካላትም ተዋሐስያኑ የሚጠቃበት ዋና ዋና ተዛማጅ ምክንያቶች ምን እንደሆኑ ለመለየት እንደሚያስችል ይጠበቃል፡፡

የጥናቱ ሂደት በዝርዝር: ውድ ለቲቢ ምርመራ የመጡ ታካሚ ፣ በጥናቱ ለመሳተፍ ከተስማሙ የስምምነት ውሉን እንደሚፈርሙና ወይም የጣት አሻራዎን እንዲሰጡ፤ ከዚህ ቀጥሎ የተዘረዘሩ መረጃዎች እንዲሞሉ በትህትና እንጠይቃለን፡፡

- የርስዎን ማኅበራዊ ሁኔታን የሚመለከቱ መረጃዎች ይጠየቃሉ፡፡
- ቃለ መጠይቁን ለማካሄድ በአማካኝ 10 ደቂቃዎች ብቻ ይሰጡናል፡፡
- ለቲቢ ምርመራ የሚሰጡትን የአክታ ናሙና ከቲቢ ምርመራ በኋላ ለጥናቱ እንወስዳለን፡፡

- ናሙናውም ከሳምባ ነቀርሳ ጋር ተመሳሳይ በሆነ ደረጃ በሳምባ ላይ ጉዳት ለማምጣት ምክንያት የሆኑት የክራፕቶስፖሪዲየም ኦኦሲት የተሰኙ ረቂቅ መኖራቸዉን ለማረጋገጥ በጅማ ዩኒቨርሲቲ የህክምና ማእከል በማይኮ ባክቴርዮሎጂ የምርምር ክፍል ምርመራ ይደረግበታል፡፡

በጥናቱ በመሳተፍዎ የተነሳ የሚመጣ ስጋት እና ጉዳት: በዚህ ጥናት ተካተው የአከታ ናሙናዎ በመስጠትዎ የሚደርስብዎ አንዳችም ጉዳት እና ሥጋት የለም አይኖርምም ::

በጥናቱ በመሳተፍዎ ሊያገኟቸው የሚችሉት ጥቅሞች: በዚህ ጥናት ውስጥ በመሳተፍዎ በጥሬ ገንዘብ የሚደረግ ምንም አይነት ጥቅም የለም አይኖርምም :: ነገር ግን ለህክምናው ከሰጡት አከታ ውስጥ የክራፕቶስፖሪዲየም እና/ወይም ቲቢ በሽታ አምጭ ተህዋሲያኑ በምርመራው በጥናቱ ወቅት ቢገኝ ወጤቱን እርስዎን ወደ መረመረዎ ሐኪም በመላክ ተጨማሪ ሕክምና እና ክትትል እንዲያገኙ ይደረጋል፡፡

የጥናቱ ውጤት ምስጢራዊነት: በጥናቱ የተገኙ ማንኛውም መረጃዎች ምስጢራዊነታቸዉ በማንኛውም ጊዜ የተጠበቀ ይሆናል የጥናቱ መረጃዎች በሙሉ የተቀመጡት ለጥናቱ ተብሎ በሚሰጠው የሚስጢር ቁጥር ሲሆን ጥናቱን ከሚያካሂዱት ባለሙያዎች በስተቀር ሌላ አካል አያውቀውም ፡፡ የጥናቱን ተሳታፊ ማንነት በሚገልጥ መልኩ የተዘጋጀው መረጃ የጥናቱ ተሳታፊ በፊርማዉ የተረጋገጠ ፈቃድ ሳይሰጥ ይፋ አይደረግም:: ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢወጣ ወይንም በመገናኛ ብዙሃን ቢነገር የጥናቱ ተሳታፊ ስም በፍጹም አይጠቀስም፡:

በጥናቱ ውስጥ የፈቃደኝነት ተሳትፎ እና ያለመሳተፍ መብት: በዚህ ጥናት ዉስጥ የሚኖርዎት ተሳትፎ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ሲሆን በጥናቱ ውስጥ ከተካተቱ በኋላ በማንኛውም ጊዜ ይህንን ጥናት ማቋረጥ መብትዎ ነው፡፡ በጥናቱ ባለመሳተፍዎ ምክንያት በአሁኑ ወይም ለወደፊት በሚደረግልዎ የህክምና እርዳታዎች ላይ ምንም ዓይነት ተፅዕኖ አይኖረውም፡፡ ስለጥናቱ ማንኛዉም ጥያቄ ወይም ቅሬታ ቢኖርዎ የሚከተሉትን የስልክ ወይም የኢሜይል አድራሻዎች በመጠቀም የጥናቱን ባለቤቶች ጠይቀው መልስ ማግኘት ይችላሉ ::

የጥናቱ ባለቤቶች	**የተንቀሳቃሽ ስልክ ቁጥሮች**	**ኢሜይል**
ı አቶ ጳውሎስ ፍሥሓ 2 ዶ/ር ዓለም-ሰገድ አብዲሳ 3 ዶ/ር ኦይስተይን ኤች ጆህንሰን 4 እጩ-ዶ/ር ዋቅጅራ ከበደ 5 ዶ/ር ዘለቀ መኮነን 6 ዶ/ር ገመዳ አበበ	… +251 913 91 45 71 … +251 931 05 71 95 … +251 932 51 46 19 … +251 911 37 83 86 … +251 917 76 54 27 … +251 911 99 12 85	… egullego7@gmail.com … alemseged.abdissa@gmail.com … haarklau@gmail.com … wakjirakebede@yahoo.com … zeleke.mekonnen@ju.edu.et … moa.ayana@gmail.com

Annex-III Consent Form

A. Consent Form -English Version

Code (starts from 001 and make continuous**):** _____

I hereby undersigned, have been informed about the study that plans to assess the prevalence of respiratory cryptosporidium infection in PTBS at JUMC. I confirmed that the objective of the study has been explained to me clearly. Having understood the entire objective and merits of the study, I have decided to participate in this study. I have been also informed that all the information contained within the questionnaire is to be kept confidential, my right to keep hold of information, decline to cooperate and make myself withdraw from the study at any time of during study. It is, therefore, with full understanding of the situation that I gave the informed consent or thump-print voluntarily to the data collectors /researcher to collect my sputum sample for the investigation.

Agree----------Not agree----------

Therefore I gave my consent/ Assent or thump-print freely for my participation in this study.

Name of data collector ---------- -Signature ----------Date----------

Name of investigator----------Signature----------Date----------

Date of data collection----------

B. Consent Form -Afaan Oromo version

Lakkoofsa hirmaataaf kenname ……

Maqaa hirmaataa----------

Yommuun qorannoo kana irratti hirmaadhu afaan naaf galuun natti himameera ykn naaf ibsameera. Faayidaa qorannoo kanaatis " Respiratory cryptosporidium infection in pulmonary tuberculosis suspects" naaf galeera. Waa'ee dhibee sombaa akkan gaafatamuu akkitaan akka kennamu naaf himameera. Odeeffannoon qorannoo kana irraa argamu hunduu iccitiin akka kaa'amus irratti walii galleerra. Qorannoo kana hirmaachuu yoon hinbarbaadne ykn yoon addaan kute ammas ta'ee fulduraaf fayyadamummaa kiyyarratti rakkoo tokkoollee akka hin uumnee naaf himameera.

Nan barbaada---------- hin barbaadu----------

Maqaa dhukkubsataa----------mallattoo----------guyyaa----------

Maqaa qo'ataa---------- mallattoo----------guyyaa----------

C. Consent Form-የአማርኛ ግልባጭ

የተሳታፊው መለያ ቁጥር (ከ001 ጀምሮ በተከታታይ ይጻፋል)_____

እኔ ከዚህ በታች ስሜ የተገለጸው እና ፊርማዬን ያኖርኩት ወይም የጣት አሻራዬን ያስቀመጥሁት የጥናቱ ተሳታፊ " የትንፈሳ ከራይፐቶስፖሪዲዮሲስ በሳንባ ነቀርሳ እንደ ተጠቁ ተጠርጥረዉ በጅማ ዩኒቨርሲቲ የህክምና ማእከል በሚመረመሩ ታካሚዎች ውስጥ " ተብሎ ሊጠና በታሰበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ አግኝቻለሁ::የጥናቱንም ዓላማ ተገልፆልኝ በደንብ ከተረዳሁ በኋላ በጥናቱ ለመሳተፍ መወሰኔን አረጋግጫለሁ:: ስለሆነም ለጥናቱ የሚያስፈልጉ መረጃዎችን እና ለቲቢ ምርመራ የምሰጠውን የአክታ ናሙና ለዚህ ጥናት ምርመራ እንደሚውል ተረድቼ ፈቅጃለሁ:: በተጨማሪም በጥናቱ ቃለ መጠይቅ ጊዜ የሚወሰዱ ማናቸውም መረጃዎች በሚስጢር እንደሚያዙ እና የሚጠየቀውን መረጃያ ለመስጠትና በጥናቱ ያለመሳተፍ መብት እንዳለኝ እንዲሁም ሃሳብ ከቀየርኩ ከጥናቱ በማናቸውም ጊዜ ራሴን ማግለል፣ እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ አሁንም ሆነ ወደፊት የማገኛቸው የህክምና ግልጋሎቶች እንደማይጓደሉብኝ ተነግሮኛል:: ስለዚህ የጥናቱን አጠቃላይ አላማ በመረዳት በፈቃደኝነት እና ስምምነት በመፈጸሜ የአክታ ናሙና እና ለጥናቱ የሚያስፈልጉ መረጃዎችን እንዲወሰዱ ተስማምቻለሁ::

እስማማለሁ ---------- አልስማማም ----------

መረጃዉን የሚሰበስበዉ ሰዉ ስም _____ፊርማ_____ ቀን_____

ምርምሩን የሚካሂደዉ ሰዉ ስም _____ፊርማ_____ ቀን _____

የጥናቱ መረጃ የተሰበሰበበት ቀን ----------

Annex-IV Questionnaire

A. Questionnaire-English version

A questionnaire prepared for study participants took part in a research entitled Respiratory Cryptosporidium infection in pulmonary tuberculosis suspects at Jimma University Medical Center, Southwest Ethiopia, 2017.

I. Socio-Demographic and Economic Characteristics

S.NO	Questions	Responses	Remark
01	Identification of the participants	1. Card No_____ 2. Lab Code: _____	
02	Age	Put age in year----------	
03	Sex	1. Male 2. Female	
04	Residence	2. Rural 2. Urban	
05	Educational status?	1. Illiterate 2. Can read and write 3. First cycle (Grade 1-4) 4. Second cycle (Grade 5-8) 5. High school & preparatory (Grade 9-12) 6. College and above	

06	Occupation kind?	1. Farmer 2. House wife 3. Student 4. Daily laborer 5. Government/NGO employee 6. Merchant 7. Others (specified as)----------	
07	Family average monthly income birr?	1 ≤ 500 2 501-1000 3 1001-1500 4 1501-2000 5 2001-3000 6 3000 – 4000 7 4000 – 5000 8 > 5000	

II-Underlining Conditions or clinical features to Respiratory Cryptosporidium infection

S.N$_O$	Questions	Responses	Remark
01	Do you have Cough?	1. Yes 2. No	
02	If yes to Qn. 1 duration of cough?	1. 2 to 4 weeks 2. 4 to 6 weeks 3. >6weeks	

03	Weight loss?	1. Before 6 weeks ----------kg 2. Currently ----------kg 3. Yes 4. No	
04	Difficulty in breathing ?	1. Yes 2. No	
05	HIV status?	1. Positive 2. Negative	
06	Were tubercle bacilli detected in the sputa?	1. Yes 2. No	
07	Were Cryptosporidium oocysts detected in the sputa?	1. Yes 2. No	
08	Any episodes of diarrhea?	1. Yes 2. No	
09	Close proximity to animal (e.g., cattle, sheep, chicken) and /or their dung?	1. Yes 2. No	

Thank you!

B. Questionnaire-Afaan Oromo Version

Yuunivarsiitii Jimmaatti

Dhaabbataa Saayinsii fayyaa fi iskuuli barnoota laaboraatoori

Kiraayiptoosporiidiyoosisii cawwee sombaatiin akka qabaman tilmaamamee hancuufni isaanii qoratamee hospitaala jiddu gala fayyaa yuunivarsitii jimmaatti warra wal'aanaman keessaa mata duree jedhun qorannoo qophaa'eirratti wal'aanamtoota hirmaatanif gaafanno qophaa'e; kibba lixaa Itoophiyaa.

Maqaa raga gaafataa---------- Mallattoo ----------

Maqaa nama qorannoo adeemsisuu ---------- Mallattoo ----------

Guyyaa ragaan itti gaafatame----------

Lakkaddaa (guca walii galtee irra kan jiru fayyadamaa) ----------

I-Ragaalee Hawaasaa fi Hariiroo Qaban

T/L	Gaaffii	Deebii	Yaada
01	Eenyummaa dhukkubsata	1. Lakk. Kaardii ..... 2. Lakk. Laaboraatoorii.....	
02	Umurii	Waggaa dhan qofa haaibsamu _____	
03	Saala	1. dhiira 2. Dhalaa	
04	Bakka jireenyaa	1. Baadiyyaa 2. Magaalaa	
05	Sadarkaa barnootaa	1. Barreessuu fi dubbisuu kan hin dandeenye 2. Barreessuu fi dubbisuu kan dandeessu 3. Sadarkaa 1ffaa (kutaa 1-4 kan baratte) 4. Marsaa 2ffaa (kutaa 5-8 kan baratte) 5. Sadarkaa 2ffaa fi Qophaa'ina (kutaa 9-12) 6. Koolleejjii fi isaa ol	
06	Haalahojii	1. Qotebulaa 2. Giiftii manaa 3. Barattuu 4. Hojjetaa guyyaa 5. Hojjetaa mootummaa/Hojjeeta dhaabamit-mootummaa keessa kan hojjetu 6. Daldaltuu 7. Kanbiraa (haaibsamu) _____	
07	Galiimaatiiji'aan (Qarshiidham)	1. ≤ 500 2. 501-1000 3. 1001-1500 4. 1501-2000 5. 2001-3000 6. 3000 – 4000 7. 4000 – 5000 8. > 5000	

II-Ragaalee dhukkuba waliin walqabatan

T/L	Gaaffii	Deebii	Yaada
01	Siqofaasisaa?	1. Eeyyee 2. Lakki	
02	Yoo lakk 01f eeyyee ta e yoomii jalqabeeti?	----------	
03	Ulfaatinni keessan hir'atee?	1 Eeyyee 2. Lakki	
04	Affuurri isin hanqataa?	1 Eeyyee 2. Lakki	
05	Haala Kiriptoo	1 Eeyyee 2. Lakki	
06	Dhibee Sombaa (PTB)	1 Eeyyee 2. Lakki	
07	HIV Eedsii	1 Posatiivii 2 Nagatiivii	
08	Siteessisee beekaa?	1.Eeyyen 2.2 Hin qabu	
09	Bobbaa beeyladaa tuqaniibeekuu?	1. Eeyyen 2 .Miti	

Galatoomaa!

C. Questionnaire-የአማርኛው ግልባጭ

በጅማ ዩኒቨርሲቲ ጤና ተቋም የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ቤት

"የትንፈሳ ክራይፕቶስፖሪዲዮሲስ በሳንባ ነቀርሳ እንደተጠቁ ተጠርጥረዉ በጅማ ዩኒቨርሲቲ የህክምና ማእከል በሚመረመሩ ታካሚዎች ውስጥ" በሚል ርዕስ በተዘጋጀ ጥናት ላይ ተሳታፊዎች የተዘጋጀ መጠይቅ፤ ደቡብ ምዕራብ ኢትዮጵያ 2017፡፡

የመረጃ ጠያቂዉ ስም---------- ፊርማ ----------

ጥናቱን የሚያካሂደዉ ሰዉ ስም----------ፊርማ ----------

መረጃዉ የተጠየቀበት ቀን ----------

መለያ ቁጥር (በስምምነት ዉል ቅጽ ላይ ያለዉን ይጠቀሙ) ----------

I-ማኅበራዊ እና ሌሎች ተዛማጅነት ያላቸዉ መረጃዎች

ተ.ቁ	ጥያቄዎች	መልሶች	ምርመራ
01	የጥናቱ ተሳታፊ መለያ ቁጥር	1 ካርድ ቁጥር---------- 2 የላቦራቶሪ ኮድ----------	
02	እድሜ	በዓመት ብቻ ይገለፅ----------	
03	ጾታ	ነ. ወንድ 2 ሴት	
04	መኖሪያ ቦታ	ነ. ገጠር 2.ከተማ	
05	የትምህርት ሁኔታ	ነ. መጻፍና ማንበብ የማይችሉ 2. መጻፍና ማንበብ የሚሉ 3. አንደኛ ደረጃ(ከ1-4ክፍል) 4. ሁለተኛ ሳይክል (ከ 5-8ክፍል) 5. ሁለተኛ ደረጃና መሰናዶ (ከ 9-12) 6. ኮሌጅና ከዚያ በላይ	
06	የሥራ ሁኔታ	ነ. ገበሬ 2. የቤት እመቤት 3. ተማሪ 4. የቀን ሠራተኛ 5. በመንግስት/ኢመንግስት ድርጅት ዉስጥ የሚሠሩ 6. ነጋዴ	
07	የቤተሰብ አማካኝ የወር ገቢ (በብር)	1. ≤ 500 2. 501-1000 3. 1001-1500 4. 1501-2000 5. 2001-3000	

		6. 3000– 4000 7. 4000– 5000 8. > 5000	

II-ከበሽታውጋርተያያዥነትያላቸዉ መረጃዎች

ተ.ቁ	ጥያቄዎች	መልሶች	ምርመራ
01	ሳልያስልዎታል?	ነ አዎ 2 የለም	
02	ለቁጥር 01 አዎ ከሆነ ከመቸ ጀምሮ	ነ ከ2 እስከ 4 ሳምንት 2 ከ4 እስከ 6 ሳምንት 3 ከ6 ሳምንታት በላይ	
03	ክብደት ቀንሰዋል ?	1 ከ 6 ሳምንታት በፊት -------ኪ/ግ 2 የአሁን ክብደት ----------ኪ/ግ 1 አዎ 2 የለም	
04	ትንፋሽ ያጥረዎታል ?	1 አዎ 2 የለም	
05	በአከታው ውስጥ ከራይፐቶስፖሪዲየም ኦኦሲስት ተገኝቷል ?	1 አዎ 2. የለም	
06	በአከታቸው ውስጥ የሳንባ ቲቢ ተገኝቷል ?	ነ አዎ 2.የለም	
07	ኤች ኤይ ቪ አለባቸው ?	1 ፖሰቲቭ 2 ነጋቲቭ	
08	ተቅማጥ ያስቀምጥዎታል?	1 አዎ 2 የለም	
09	ከቤት እንስሳቱ ጋር ወይም ከጽዳጃቸውጋር ንክኪ አለዎት ?	1 አዎ 2 የለኝም	

እናመሰግናለን!

Annex-V. Declaration sheet

I hereby the undersigned, declared that this thesis finding is my own original work and has never been presented for any degree in Jimma University or any other higher education institutions in Ethiopia. I also declared the properly acknowledgement of all material resources used for this thesis work.

Principal investigator

1. Paulos Fissiha (BSc, Jimma University)

   Signature ----------Date----------

Co-investigators:

1 Alemseged Abdisa (PhD, Jimma University)
Signature ----------Date----------
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   Signature----------Date----------
3. Wakjira Kebede (MSc, PhD scholar, Jimma university)
   Signature----------Date----------
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   Signature----------Date----------
5. Gemeda Abebe (PhD, Jimma university)
   Signature----------Date----------

APPROVAL SHEET OF THESIS

As a member of the Board of Examiners of the master of science thesis open defense examination, I certify that I have read, evaluated the thesis prepared by Paulos Fissiha, and examined the candidate as well. I recommended that the thesis be accepted by fulfilling the thesis requirements for the degree of Masters of Science in Medical Microbiology.

2. Internal examiner: _____

   Date._____ Signature _____

4. External examiner: _____

   Date._____ Signature _____