PREVALENCE, RISK FACTORS AND TRACHOMA CAUSING SPECIES CIRCULATING IN EAST POKOT, BARINGO COUNTY, KENYA

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Prevalence, Risk Factors and Trachoma Causing Species Circulating in East Pokot, Baringo County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature……..Date……

Joan Wangui Kamau

This thesis has been submitted for examination with our approval as University Supervisors.

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JKUAT, Kenya

DEDICATION

It is with humility and honor that I dedicate this project to the Almighty God for his mercy, grace, love, wisdom and for giving me the strength to complete this study.

This work is also dedicated to my family. You have been my source of inspiration and encouragement.

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ABBREVIATIONS AND ACRONYMS

AMREF	African Medical and Research Foundation
GET	Global Elimination of Trachoma
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KIHBS	Kenya Integrated Household Budget Survey
KNBS	Kenya National Bureau of Statistics
MOH	Ministry of Health
NUITM	Nagasaki Institute of Tropical Medicine
PCR	Polymerase Chain Reaction
SAFE	Surgery Antibiotics Facial cleanliness and Environmental Improvement
UIG	Ultimate Intervention Goal
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

ABSTRACT

Trachoma is the leading cause of infectious blindness and is caused by the bacterium *Chlamydia trachomatis.* Trachoma is mainly found in the developing world among marginalized communities where there is scarcity of clean water and lack of proper sanitation and hygiene. These communities are also plagued by poor access to health care and health facilities. Trachoma has also been found to disproportionately afflict women and children in view of the fact that women are the main care givers. Trachoma was found to be endemic in six counties in Kenya including Baringo County. The Ministry of Health in Kenya has adopted the WHO SAFE or elimination of trachoma by 2020. Despite these interventions, the prevalence of trachoma is still high at 23% and 3.3% for both infectious and blinding trachoma respectively. This study investigated the prevalence and risk factors for trachoma among residents of East Pokot, Baringo County. This was a descriptive cross-sectional study, targeting 450 people of all ages living in East Pokot. The sample size was calculated using the Cochrane formula.The study objective was to determine prevalence, risk factors and Trachoma causing Chlamydia species circulating in East Pokot, Baringo County, Kenya. The study employed a multi-stage and simple random sampling procedure. Villages in target area were classified as clusters where households were selected randomly within the villages. Cluster Random Sampling was done at household level and all household members within the house were sampled. A structured questionnaire was used to collect social demographic data for each household. Conjunctival swabs were collected using a sterile swab. DNA was extracted from the eye swab and PCR analysis carried out for diagnosis of the different Chlamydia species. Data was analyzed using STATA and descriptive statistics were used to summarize demographic profiles and determine prevalence and odds ratio, multiple regression analysis to assess the independent effect of each determinant risk factor after controlling for all other factors. The significance level of this study was 5% (p=0.05). Out of the 450 samples collected, a total of 405 samples were analyzed. The prevalence of trachoma was found to be 44.44%. Of this, 14.07% (57) were confirmed PCR positive. PCR results showed dual infection: 12.28% (7) had *C. trachomatis* and *C. psittaci,* 8.77% (5) had *C. psittaci* and *C. pneumonia,* and none had *C. trachomatis* and *C. pneumonia.* None of the samples had triple infection with all the three species: *C. trachomatis, C. psittaci and C. pneumonia*. Adults aged above 9 years had higher odds of getting trachoma (OR=3.88, 95% CI 1.07-14.12, p=0.04) compared to the children aged 9 years and below. There was however no significant difference in the prevalence of trachoma between males and females (p=0.79). The findings of this study indicate that guardians and

children in East Pokot are at considerable risk of trachoma infection due to the behavioral practices and attitudes. The factors significantly associated with trachoma were found to be secondary education (p=0.036) and indigenous religion (p=0.0258). A total of 180 (44.44%) out of the 405 participants were diagnosed as clinically Trachoma positive. Of these, 25 (13.89%) tested laboratory positive for Chlamydia. On the other hand, 225 (55.56%) were diagnosed as clinically Trachoma negative and of these 20 (8.89%) tested chlamydia negative in the laboratory. Therefore, the overall concordance between the clinical diagnosis and laboratory diagnosis was 29.94%Results showed that active trachoma is still a major public health concern in the study area. Health education and promotion activities for awareness creation with an aim of changing cultural perceptions and practices that contribute to trachoma transmission need to be emphasized. The community also needs to be encouraged to build and utilize latrines for human waste disposal and the County Government of Baringo should consider drilling water points to promote proper hygiene practices that will help control trachoma transmission and bring down the prevalence levels below the WHO threshold (<10 % prevalence).

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Acute infection of *Chlamydia trachomatis* begins in childhood and advances over the years with repeated infections. If left untreated trachoma infection causes chronic inflammation of the conjunctiva, the inner eye lid, causing scarring and irritation which leads to shrinkage of the eyelid and eye lashes causing them to turn inwards abrading the cornea. This is referred to as trichiasis and may lead to cornea opacity and eventual blindness during mid-life (Sommer et al., 2014). Active trachoma is mostly seen in young children aged 1-9 years. Cicatricial (scarring) complications and blindness are seen in late childhood and adulthood (Mariotti *et al*., 2009).

Trachoma is an eye infection caused by the bacterium *Chlamydia trachomatis* which is an obligate intracellular parasite. It is a contagious disease spread by both direct and indirect contact with an infected person's eyes or nose. The housefly, *Musca sorbens,* is the transmission vector for trachoma, therefore activities and distribution of the housefly will also affect prevalence (Ramesh *et al*., 2013). *Chlamydia trachomatis* has a number of serotypes that cause different diseases, but serovars A, B, Ba and C cause trachoma (Derrick, Roberts, Last, Burr, & Holland, 2015). Studies have shown that more than one species of chlamydiaceae may be responsible for causing trachoma eye infection. Therefore, the probability that multiple strains of chlamydiaceae are associated with trachoma would mean a re-evaluation of current approach of the study and treatment including vaccines for trachoma (Dean, Kandel, Adhikari, & Hessel, 2008).

This may further explain why in endemic areas where there is sufficient uptake of the SAFE strategy there is a high rate of trachoma relapse among the patients who have received surgery for trachiasis or antibiotics to treat infection. The failure to diagnose active trachoma cases may also be attributed to detection of only one species of chlamydia (Dean et al., 2008). In Nepal a study reported the first evidence of involvement of multiple chlamydiaceae species in ocular trachoma. The bacteria detected include *C. trachomatis*, *C. psittaci* and *C.pneumoniae*. A further 35% of the infected individuals had mixed infections which consisted primarily of two chlamydiae species. Although the data they found was specific to Nepal, it is likely that multiple species of chlamydiae are involved in trachoma in endemic regions such as East Pokot, Baringo County (Dean et al., 2008).

Approximately 21 million people currently have active trachoma (conjunctival inflammation), the vast majority being in Africa (Frick *et al*., 2003). 98% of trachoma is found in developing countries, primarily in sub-Saharan Africa and the Middle East with considerable pockets of endemicity in Asia, Mexico and Latin America (Ngondi *et al*., 2008). Trachoma is believed to be endemic in 57 countries with more than 80 percent of the burden of active trachoma concentrated in 14 countries in Africa where there is need for prompt action. It is estimated that every 15 minutes someone goes blind from trachoma (INSight, 2011). Globally, there is an estimated loss of $2.9 billion in productivity each year due to trachoma and blinding trachoma (Frick *et al*., 2003; Habtamu *et al*., 2015). This can have a devastating impact on whole families and communities at large (Mabey, 2009). Although trachoma has virtually disappeared in developed countries, possibly due to improved living and hygiene standards, it continues to plague the developing countries due to poverty, crowded living conditions and lack of proper sanitation. Communities with trachoma are largely underprivileged and most frequently

located in remote areas in developing countries (Habtamu *et al*., 2015). As trachoma besets the most deprived people in the world, it leads to disability, dependency and greater poverty (Oswald, 2017).

Trachoma is endemic in six counties in Kenya: Samburu, Narok, West Pokot, Kajiado, Baringo and Meru. Surveys have shown blinding trachoma to be a public health problem in four counties: Samburu, Narok, West Pokot and Kajiado (Karimurio *et al*., 2006). Valid and reliable data on the distribution of active trachoma within a country, based specifically on the district prevalence of the disease is crucial to the success of GET 2020, an alliance formed by WHO in 1997 (Polack *et al*., 2005).

1.2 Statement of the problem

Trachoma is a public health concern in the world as it is a leading infectious cause of blindness and many more people are at risk of infection and it is estimated to cause a loss of $2.9 billion in productivity globally (Mariotti *et al*., 2009). WHO estimates that 98% of trachoma is found in developing countries, primarily in sub-Saharan Africa and the Middle East, with considerable pockets of endemicity in Mexico, Latin America and Asia. In Kenya, trachoma accounts for 19% of the blindness making it the second leading cause of avoidable blindness (Karimurio *et al*., 2006). Trachoma was found to be endemic in six counties (former districts), namely; Samburu, Narok, West Pokot, Kajiado, Baringo and Meru North where it was a public health concern causing trachoma inflammation in children aged 9 years and below trichiasis which leads to blindness in adults aged 15 years and above (Karimurio *et al*., 2006). A national blindness survey conducted in the 1980's indicated that trachoma may still be endemic in 18 out of the 73 districts in

the country. The Pokot are among the most affected communities in Kenya (KNBS, 2009). A national blindness survey report showed that fifty percent of children aged less than ten years had active trachoma compared to other communities with a prevalence of less than twenty five percent (Karimurio *et al.*, 2006). This can be attributed to the nomadic lifestyle of the Pokot as well as closely linked to environmental practices that support the life cycle of the house fly, which is the main transmission vector for the disease.

Efforts in trachoma elimination have been focused on the use of antibiotics to treat infection and surgery for trichiasis with less emphasis on environmental improvement and facial cleanliness to reduce transmission of organism, contributing to increased prevalence of trachoma (WHO, 2003). Trachoma accounts for 19% of blindness and is the second leading cause of avoidable blindness in Kenya (Karimurio *et al.*, 2006). Therefore, it is important to determine the prevalence of trachoma in Kenya to facilitate support visual disabilities in terms of preventative and curable treatments. This study seeks to fill this gap by determining the prevalence and factors associated with trachoma in East Pokot, Kenya.

1.3 Justification of the study

Global elimination of trachoma is a priority on the World Health Organization elimination agenda. This is through the component that aims at eliminating both infectious and avoidable blindness by 2020. This may not be realized if only the clinical aspects of trachoma are looked into while other underlying factors influencing the disease are over looked. For a lasting and sustainable solution,

there needs to be a holistic approach which incorporates facial hygiene, antibiotics, environmental sanitation and surgery.

As a method of eliminating trachoma in Kenya, the SAFE strategy has been adopted by the Ministry of Health with support from such organizations such as Sightsavers, The Queen Elizabeth Diamond Jubilee Trust, The Fred Hollows foundation AMREF and UNICEF (Health., 2016; Ngondi *et al*., 2008). Despite the efforts put in fighting the disease, trachoma has persisted to be a public health concern in Kenya and more so among the Pokot community in East Pokot, Baringo County (Karimurio *et al*., 2006). Additionally to the studies carried out on the clinical aspects of trachoma, the study provides valuable data in understanding the association of risk factors and how this hinder the efforts in elimination of trachoma in East Pokot, Baringo County. East Pokot was chosen as a study area based on the high burden and prevalence of trachoma cases as identified by the MOH trachoma control programmes (Health., 2016; Karimurio *et al*., 2006).

It is anticipated that the findings of this study will add to the knowledge on molecular epidemiology of trachoma in East Pokot, Baringo County, Kenya. Molecular epidemiology studies are essential before new trachoma control projects are funded and existing ones continued. The results of this study will help establish risk factors and circulating speciesof trachoma in East Pokot at the sub-district level with certainty thus ensuring trachoma endemic areas in East Pokot are not missed out and non-endemic areas are not included in mass drug treatment which takes place nearly annually. Trachoma is an infectious disease and hence detailed data on circulating species and associated risk factors is a valuable tool in the control and development of public health policies for trachoma. The data on circulating species and risk factors is useful in assessing the magnitude of the

disease, determining priority areas, monitoring changes and advocacy. The lack of data in many countries remains an obstacle to trachoma control efforts.

1.4 Research questions

1. What is the prevalence of trachoma in East Pokot, Baringo County?
2. What are the Trachoma causing Chlamydia species in East Pokot, Baringo County?
3. What are the risk factors associated with trachoma in East Pokot, Baringo County?

1.5 Objectives

1.5.1 General objective

To determine the prevalence, risk factors and Trachoma causing Chlamydia species circulating in East Pokot, Baringo County.

1.5.2 Specific objectives

1. To determine the prevalence of trachoma in East Pokot, Baringo County.
2. To determine the Trachoma causing Chlamydia species in East Pokot, Baringo County.
3. To establish the risk factors associated with trachoma infection in East Pokot, Baringo County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of trachoma

Trachoma is a chronic disease that slowly and painfully causes blindness. The loss of vision from trachoma is irreversible (Burton *et al*., 2015). Many people in endemic areas have clinical signs of active trachoma (follicular trachoma, inflammatory trachoma; TF/TI), follicles and papillae on the conjunctiva epithelium of the upper eyelid which occurs mostly in children aged 1-9 years. These symptoms are as a result of inflammatory response following infection with *C.trachomatis* (Ngondi *et al.*, 2008). Trachoma is highly contagious and is spread by direct contact with an infected person's eye and nasal secretions by hand, fomites such as towels and eye seeking flies (WHO, 2003).

Risk factors associated with trachoma include poor sanitation, crowded living conditions, lack of enough clean water and toilets also increase transmission of trachoma (Mahande *et al*., 2012). In most cases the facial cleanliness and environmental improvement parts of the SAFE strategy are not adopted causing reinfection with trachoma. Climatic factors may also have an impact on the epidemiology of trachoma where areas with low rainfall have a higher prevalence of active trachoma (Ramesh *et al*., 2013). According to a study done in Tanzania, smoke by exposure to indoor cooking fire is also a likely risk factor for trachoma (Zambrano *et al*., 2015). Women are 3-4 times at a higher risk of trachoma infection and blindness compared to men (Karimurio *et al*., 2006). Environmental risk factors influencing the transmission of the disease include: poor hygiene, crowded households, water shortage, keeping cattle close to the

home, inadequate latrines and sanitation facilities which promote effective breeding sites for eye-seeking flies (Emerson *et al*., 2005). These factors are common in many rural and semi-arid areas of Kenya such as Baringo. Climatic factors affecting trachoma directly or indirectly have also been of growing interest (WHO, 2003).

2.2 Cause and transmission of trachoma

Chlamydia trachomatis has a number of serotypes that cause different diseases, but serovars A, B, Ba and C cause trachoma (Derrick *et al*., 2015). Recent studies have shown that more than one species of chlamydiaceae may be responsible for trachoma eye infection and have highlighted the heterogeneous response to mass treatments by communities where prevalence is lowered in some communities while in some communities prevalence quickly resumes to pretreatment levels (Hu *et al*., 2013). Consequently, the probability that multiple strains of chlamydiaceae are associated with trachoma would mean a re-evaluation of current approach to treatment including vaccines for trachoma (Dean *et al*., 2008). In Nepal a study reported the first evidence of involvement of multiple chlamydiaceae species in ocular trachoma. The bacteria detected included *C. trachomatis, C. psittaci* and *C.pneumoniae*. A further 35% of the infected individuals had mixed infections which consisted primarily of two chlamydiae species. It is likely that multiple species of *Chlamydiae* are involved in trachoma in endemic regions such as East Pokot, Baringo County (Dean *et al*., 2008). The housefly, *M. sorbens,* is the transmission vector for trachoma and for that reason its activities and distribution affect prevalence of trachoma. Trachoma is highly contagious and is mainly transmitted by direct contact with an infected person's eye and nasal secretions by hand, fomites such as towels and eye seeking flies (Last *et al.*, 2014).

2.3 Pathology of trachoma

Active trachoma is more common in children under 10 years, while blinding trachoma affects adults 18 years and older (Bailey, 1991). Young children, below the age of 10 years, are the main reservoirs of *Chlamydia trachomatis* (West *et al*., 1991). Acute infection of *Chlamydia trachomatis* begins in childhood and advances over the years with repeated infections. If left untreated trachoma infection causes chronic inflammation of the conjunctiva, which is the inner eye lid, it causes scarring and irritation which leads to shrinkage of the eyelid and eye lashes to turn inward abrading the cornea. This is referred to as trichiasis and may lead to cornea opacity and eventual blindness during mid-life (Sommer *et al*., 2014). Infectious trachoma (active) initially presents as a red eye-itching, redness and pain characterized by mixed follicle trachomatous occurring in the upper tarsal conjunctiva and associated with mucopurulent discharge. Blinding trachoma (chronic) is prevalent in middle age adults and is characterized by broad confluent scars on the conjunctiva, trichiasis, distichiasis, corneal vascularization and cicatricial entropion, corneal opacification and a dry eye (Derrick *et al*., 2015). These two states of the disease, trachoma folliculitis and cornea opacity are shown in the diagram below figure 2.1.

Figure 2.1: Active trachoma showing follicles on the left and chronic trachoma showing cornea opacity on the right.

2.4 Risk factors for active trachoma

Trachoma is the leading cause of infectious blindness in the world. In the developing world, blinding trachoma continues to be a huge public health concern due to poor living conditions which facilitate continuous transmission of *Chlamydia trachomatis* among family members. There are individual and household risk factors associated with active trachoma in children and trichiasis in adults. Preschool-aged children in trachoma endemic areas can have prevalence rates as high as 60-90% for active (inflammatory) trachoma (Schwab *et al*., 1995). Identification of risk factors is crucial for planning and implementing effective trachoma control programs.

2.4.1 Availability of clean water

Studies have shown that poor sanitation is associated with the risk of trachoma, with hygiene conditions varying depending on availability of a convenient water

point. The long distance travelled to fetch water was found to have a positive association with active trachoma in households and among children (West *et al*., 1991. It is expected that the longer the distance travelled to fetch water, the lesser the amount of water is available for use in the household for effective sanitation (Ngondi *et al.*, 2008). It has been shown that families that have trachoma use less water daily compared to families without (West *et al*., 1991). Hygiene is not a priority where there is a limited amount of water available for use in the household (AMREF, SSI and MOH, 2004).

2.4.2 High density of Flies

Flies are the physical vectors for transmission of *Chlamydia trachomatis.* Presence of flies has been associated with trachoma. Research done by AMREF and MOH showed a positive association between fly density in the compound or household and presence of flies on children feaces with presence and severity of trachoma (AMREF and MOH, 2004). Waste that is not properly disposed increases the fly density in a compound, including presence of cow dung, defecation sites and altitude (Cumberland *et al*., 2008). The breeding ground for *M. sorbens*, eye-seeking fly, is normally exposed human feaces on the ground. Suitable construction and use of latrines is therefore necessary for the control of house fly density in the household (Emerson *et al*., 2001).

2.4.3 Cattle rearing

The presence of cattle dung attracts the housefly and acts as a breeding ground. These flies land on a child with a dirty face, acting as vector for trachoma transmission. Presence of cattle in close proximity to households increases housefly populations leading to increased transmission of trachoma (AMREF, SSI

and MOH, 2004). Studies have also shown that households that keep cattle inside the house have a significantly higher rate of trachoma compared to those that keep cattle in the compound (Taylor *et al*., 2010).

2.4.4 Latrine in the homestead

Pit latrines are important for the removal of human feaces from the ground. Pit latrines do not support the breeding of the housefly and are thus important in reducing the population of flies, fly contact and transmission of trachoma (Last, 2014). Studies have shown availability and proper use of latrines as a sustainable way of controlling fly populations in the compound (Emerson *et al.*, 2004). Presence of latrine coupled with health education was shown to significantly reduce fly population thus decreasing eye-contact and prevalence of active trachoma in children 1 to 9 years (Emerson *et al.*, 2004).

2.4.5 Facial Hygiene

It has been shown that regular and sustained face washing can lead to reduction in active trachoma (WHO, 1997). One of the main routes of transmission for trachoma is through secretions from an infected child to another person's eyes either via face towel, hands, towels, handkerchief or flies. Discharge on the face attracts flies. Children observed to have flies on the face and nasal discharge were twice as likely to have active trachoma in comparison to children without these signs (Emerson *et al.*, 2006).

Regular face washing lowers the likelihood of infected secretions been taken to another person's face (Taylor *et al*., 2010), also, frequent face washing reduces the likelihood of automatic re-infection. Children observed in homes with a clean face

were less likely to have active trachoma compared to those with unclean faces who were strongly linked with the presence of active trachoma (Solomon, 2003).

2.5 Global distribution of trachoma

The earliest references to trachoma come from China in the 27th century BC (Al-Rifai, 1988). Features of trachoma were also described in the Ebers papyrus from Egypt, 15th century BC, and epilation forceps discovered in tombs from the 19th century BC (Maccallan, 1931; Hirschberg 1982). The causative bacteria, *Chlamydia trachomatis* was not isolated until 1957 (Tan, 2004). British troops brought trachoma infection to England and Europe from Napoleon wars in the 19th century causing a major public health problem. To control the epidemic in Europe, rigorous control measures were introduced in the early 20th century. Due to improved sanitation and general living standards, trachoma has virtually disappeared in the industrialized world, but continues to be a public health problem in the developing world. Blinding trachoma remains endemic in the poorest regions of Africa, Asia, and the Middle East and in some parts of Latin America and Australia (Thylefors *et al*., 1995).

An estimated 190.2 million people are at a risk of trachomatis blindness in 41 one countries. Globally, there is an estimated loss of $2.9 billion in productivity each year due to trachoma and blinding trachoma (Mabey, 2009). WHO estimates that 98% of trachoma is found in developing countries, primarily in sub-Saharan Africa and the Middle East with considerable pockets of endemicity in Asia, Latin America and Mexico. The incidence of active trachoma on the whole is unknown, nonetheless, the prevalence of active trachoma varies between 10%-40% in some African countries and 3%-10% in several Asian countries (Frick *et al*., 2003).

2.6 Prevalence of trachoma disease in Africa

Approximately 21 million people currently have active trachoma (conjunctival inflammation), the vast majority being in Africa (Smith, 2013). Almost all of the trachoma, 98% is found in developing countries, primarily in sub-Saharan Africa and the Middle East with considerable pockets of endemicity in Asia, Mexico and Latin America (Ngondi *et al.*, 2008) (figure 2.2). Trachoma is believed to be endemic (prevalence greater than 5% in children) in 57 countries with more than 80% of the burden of active trachoma concentrated in 14 countries in Africa where there is need for prompt action (Frick *et al*., 2003).

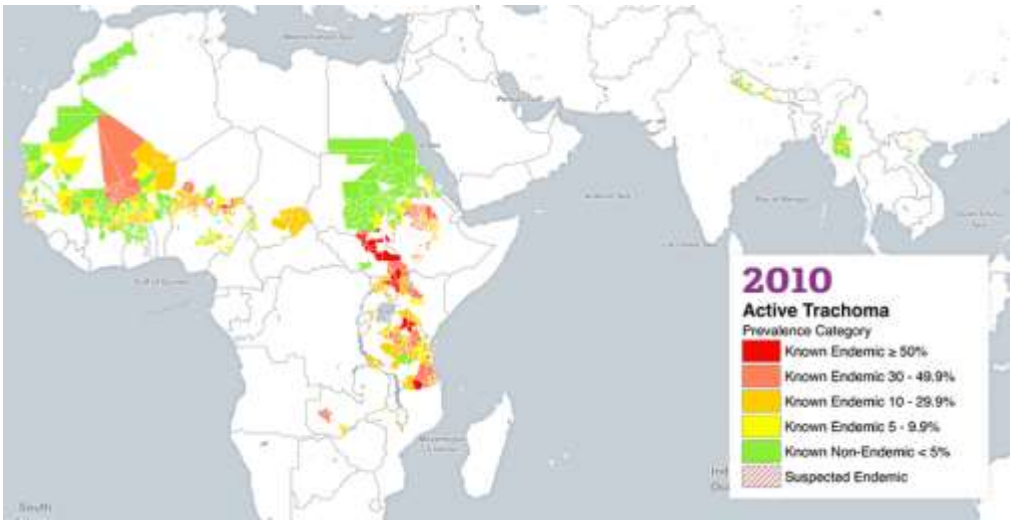

Figure 2.2: Global Atlas of Trachoma. (Source: Trachoma atlas.org)

Although trachoma has virtually disappeared in developed countries possibly due to improved living and hygiene standards it continues to plague the developing countries due to poverty, crowded living conditions and lack of proper sanitation. Communities with trachoma are largely underprivileged and most frequently

located in remote rural areas of developing countries (Habtamu *et al*., 2015). Valid and reliable data on the distribution of active trachoma within a country, based specifically the district prevalence of the disease is crucial to the success of GET 2020 an alliance formed by WHO in 1997 (Polack *et al*., 2005). There has been a significant uptake of trachoma national control programs in a number of endemic countries as a result of the 1998 WHO initiative of the global elimination of trachoma by 2020 (GET2020). These national control programs have incorporated the SAFE intervention strategy.

2.7 Prevalence of trachoma disease in Kenya

In Kenya, trachoma accounts for 19% of the blindness making it the second leading cause of avoidable blindness (Karimurio *et al*., 2006). Trachoma was found to be endemic in six counties (former districts), namely; Samburu, Narok, West Pokot, Kajiado, Baringo and Meru North where it was a public health concern causing trachoma inflammation in children aged 9 years and below trichiasis which leads to blindness in adults aged 15 years and above (Karimurio *et al*., 2006) as shown in figure 2.3. A national blindness survey conducted in the 1980's indicated that trachoma may still be endemic in 18 out of the 73 districts in the country. A national trachoma survey conducted by AMREF in 2009 found that trachoma was endemic in 6 districts out of the 18 suspected to be trachoma endemic in Kenya with a mean prevalence of 23% and 3.3% for both infectious and blinding trachoma respectively. In Kenya, like in other developing countries, trachoma is mainly found in marginalized communities with poor access to resources. Trachomatous follicular inflammation (TF) is a public health problem in Samburu with 35.0%, Narok 30.5%, Kajiado 28.1% and West Pokot 26.6%. In Meru North county seven sub-locations and nine in Baringo County had

prevalence of TF equal to or above 5%. The difference between prevalence in boys and girls was not significant in the districts (counties) surveyed except for Kajiado district where the prevalence of TF in boys was higher at 32.0% compared to girls 24.0% (p-value 0.03) (Karimurio *et al*., 2006).

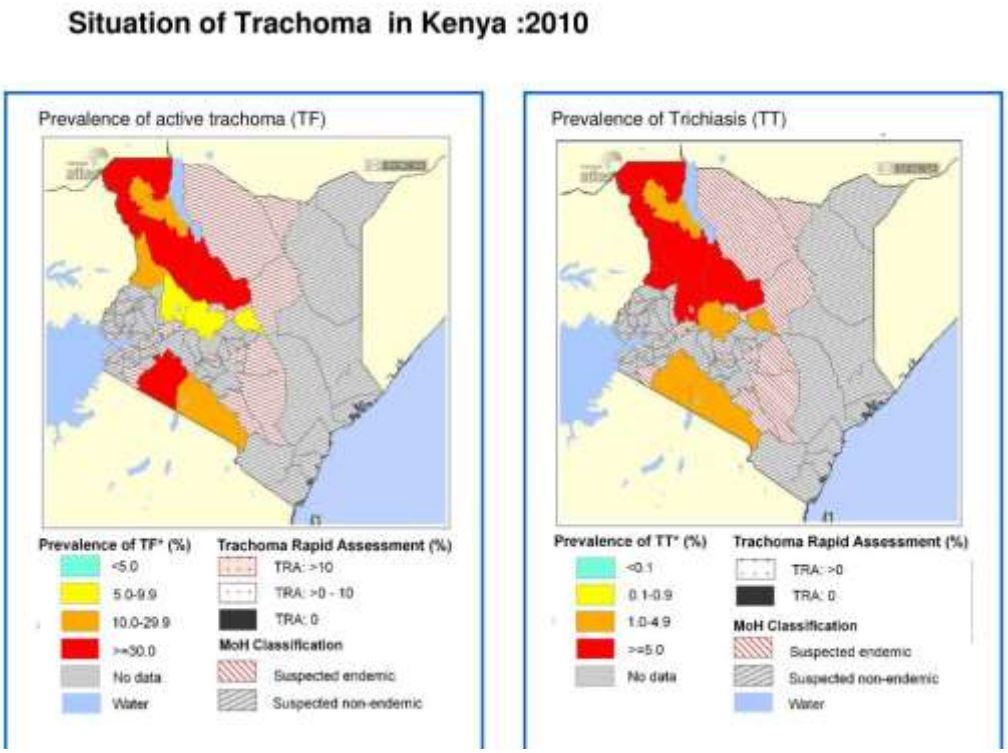

Figure 2.3: Prevalence of trachoma in Kenya

Source: (Kenya trachoma action plan 2011)

2.8 Trachoma and Women

Trachoma is an important women's health issue, it disfigures and blinds three times as many women as men, yet trachoma is both preventable and treatable

(Mahande *et al*., 2012). Traditional gender roles, especially those still practiced in many developing countries contribute to more women blinded by trachoma compared to men. Women and older girls are the primary care givers for children. Children below nine years of age are the main reservoirs for active trachoma and therefore transmission of trachoma is mainly from an infected child to the mother making women at a higher risk of trachoma infection (Last *et al.*, 2014). Studies done in trachoma endemic areas show 86% of cases of trichiasis (the stage of the diseases leading to blindness) are women. (Habtamu *et al*., 2015).

2.9 Diagnosis of trachoma

Many people in endemic areas have clinical signs of active trachoma which are follicles and papillae on the conjunctiva epithelium of the upper eyelid (follicular trachoma, inflammatory trachoma; TF/TI) and occurs mostly in children. These symptoms are as a result of inflammatory response following infection with *C.trachomatis* (Kasi *et al*., 2004). The diagnosis of trachoma is done clinically following WHO simplified trachoma grading system for clinical diagnosis of trachoma namely based on inflammation of cornea; trachomatous inflation follicular (TF), trachomatous inflammation intense (TI), trachomatous scarring (TS), trachomatous trichiasis (TT) and corneal opacity-CO (WHO, 2003). This study used the WHO simplified trachoma grading system for clinical diagnosis of trachoma. The limitation with clinical diagnosis is that it is easy to overlook asymptomatic cases or over diagnose when symptoms of another disease are very similar to target disease, recovery phase of infection can result in under diagnosis before and after treatment meaning there is a high likelihood of misallocation of treatment. PCR has a higher sensitivity compared to other laboratory techniques such as antigen detection and DNA hybridization and was used to detect the

presence of ocular chlamydia infection and can be used to further explore the relationship between infection and clinical symptoms of trachoma. PCR was important due to higher correlation between disease and infection and applicable in areas with low prevalence. PCR was useful to confirm the clinical diagnosis of trachoma and also demonstrate the presence of the agent even in the absence of overt clinical signs of active disease. PCR also presented an objective and quantifiable component into the diagnosis and epidemiological study of trachoma (WHO, 1975).

The different stages of clinical trachoma are shown in figure 2.4.

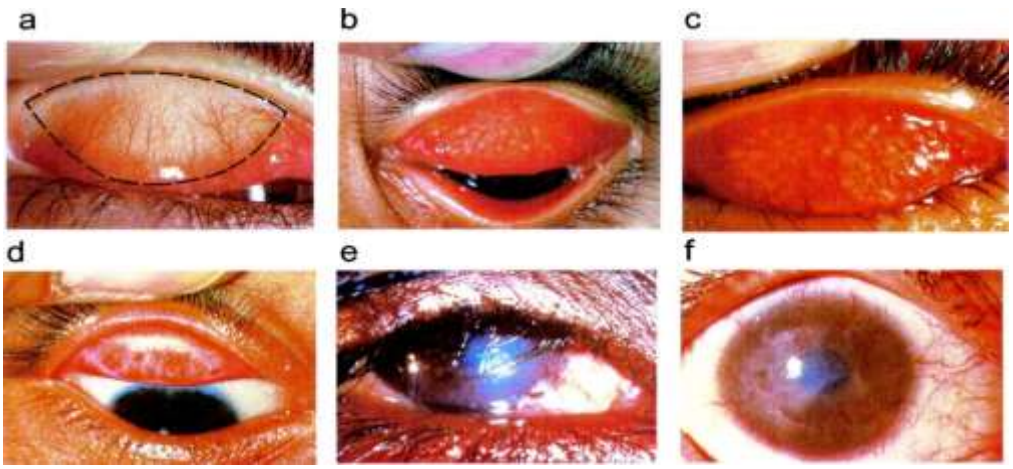

Figure 2.4: (a) Normal conjunctiva, showing area to be examined. (b) Follicular trachomatous inflammation (FC). (c) Intense trachomatous inflammation (TI). (d) Conjunctival scarring (TS). (e) Trichiasis (TT). (f) Corneal opacity (CO). (Source: WHO)

The DNA was extracted using TAKARA MightyAmp. *C. trachomatis C. psittaci* and *C. pneumonia DNA* was detected using primers of the OMP1 gene. The CT_F and CT_R, Cps_F and Cps_R and Cpn-F and Cpn_R primers generate fragments that are 461bp, 355bp and 181bp. The amplification mixture contained 5.76ul water, 10ul buffer, 2ul additive, 0.84ul primer mix and 0.4ul polymerase. The mixture was denatured for 10secs at 98ºC. The samples were amplified in 40 cycles. Each cycle consisted of denaturation at 98ºC (10sec), annealing at 60ºC (15sec) and extension at 68ºC (35sec). The product was allowed to cool and stored at 4ºC (Schachter *et al*., 1973). The amplification products were analyzed by electrophoresis in 2.5% agarose gel stained with 0.2% GelRed for 30 minutes. The gel was read using UV-camera box (C1 program. ISO=400). A band of molecular weight corresponding to 461bp, 355bp and 181bp constituted of a positive result for *C. trachomatis, C. psittaci* and *C. Pneumonia* respectively (Dean *et al*., 2008).

2.10 Control and prevention of trachoma in the world

Trachoma surveys are essential in that they provide the fundamental data for quantifying disease burden that facilitates planning, implementation, monitoring and evaluation of trachoma control programs. An alliance by WHO advocates for the use of SAFE strategy for trachoma control. This entails surgery to correct trichiasis, antibiotics to treat infection, facial cleanliness and environmental hygiene and sanitation improvement to interrupt transmission of *C. trachomatis.* (Habtamu *et al.*, 2015). While the trachoma SAFE intervention strategy has been implemented by a number of trachoma endemic countries, often only the Sanitation and/or Antibiotics components are adopted and as a result success rates are low and infection and disease often resurface (Hu *et al*., 2010). The WHO has set a target for global elimination of trachoma by 2020 and it outlines that in order

to achieve this target, 10% of endemic countries are expected to have achieved the Ultimate Intervention Goal (UIG) by 2013 and by 2016, 40% endemic countries should have met the criteria to stop large scale medicine interventions and began post-endemic surveillance thus realizing the goal of eliminating blinding trachoma as a public health program by 2020 (Burton *et al*., 2015). Antibiotics are effective in treating early cases of trachoma. Early treatment can prevent long-term complications. More advanced cases of trachoma may require surgery to reposition eyelashes that are growing inward toward the eye. This surgery can help limit further scarring of the cornea and may help improve eyesight (Hu *et al*, 2010). Corneal transplants are another surgical option if the cornea has become so clouded that vision is seriously impaired. Good hygiene, such as hand washing and face washing, has been shown to decrease the spread of trachoma (Mathew *et al*., 2009). Antibiotics are effective in treating early cases of trachoma. Early treatment can prevent long-term complications and eventual blindness (Mathew *et al.*, 2009)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in East Pokot sub-county, Baringo County which is located in mid-west part of Kenya where most of the areas are in the arid and semi-arid land (ASAL) (Figure 3.1). The county borders Elgeyo Marakwet, Nakuru, West Pokot, Turkana, Kericho and Uasin Gishu (Figure 3.2). It covers an approximate area of 11,015 km^2 with a total population of about 552,254 (KNBS, 2009). It has Lake Baringo which is one of Kenya's largest fresh water lakes which historically has been very important for fishing as well as fresh water reserve for livestock. Today the lake has two perennial rivers; Perkerra and Molo down from seven in 1970 (Johansson & Svensson, 2002). Baringo County topography is made up of plains plus some volcanic hills and valleys. The area receives an annual rainfall ranging from 200 to 700 mm, with average temperature between 20^0 C and 30^0 C. The region is very dry with no constant flowing rivers and is classified as semi-arid. Communities cover an average of 10 km seeking water. Rural households rely mostly on unimproved sources of water such as pond, dam, lake, river/stream, unprotected springs and unprotected dug wells. The poverty rate is estimated at 11.6% and access to improved sanitation is at 30% (KIHBS, 2009).

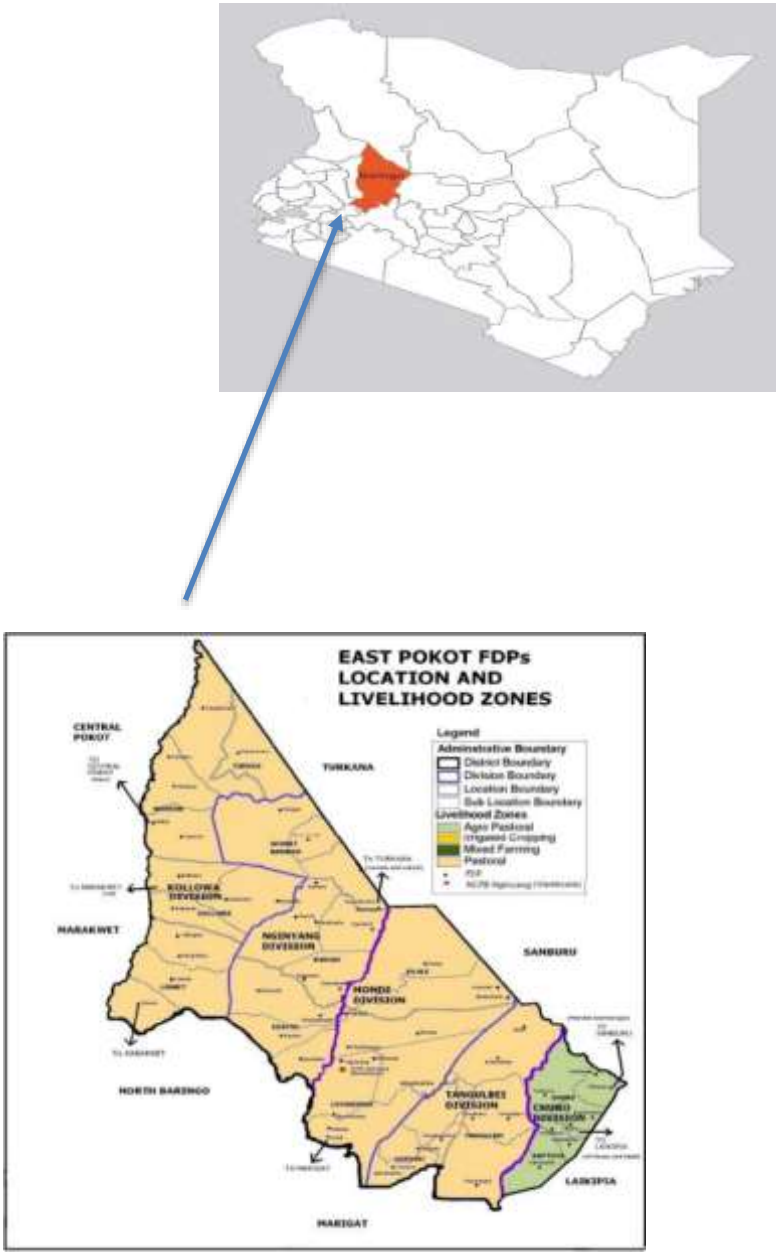

Figure 3.1: Map of East Pokot, Baringo County

Source: (Google Map, 2017)

3.2 Study design

This was a descriptive cross-sectional study. The study design enabled determination of the prevalence of trachoma and associated factors.

3.3 Study population

The study population comprised of people living in East Pokot, Baringo County who were present in their homes at the time of the study.

3.3.1 Inclusion criteria

- Individuals from randomly selected households in East Pokot, Baringo County.
- Individuals who agreed to participate in the study and children whose caregivers gave assent..
- Individuals who had not been part of a trachoma mass drug administration (MDA) in the previous two years.

3.3.2 Exclusion criteria

- Individuals not from the chosen households.
- Individuals who did not agree to participate in the study and children whose caregivers did not assent to participate.
- Individuals who had been part of a trachoma mass drug administration (MDA) in the previous two years.

3.4 Sample size determination

The sample size was calculated using Cochran's formula (Cochran, 1977) and the study assumed an approximate Trachoma prevalence of 34% (Ministry of Health, 2016).

$$n = \frac{z.p\,(1-p)}{e^2}$$

Where:

n= Estimated minimum sample size

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

d=margin of error

P= 34%

$$n = \frac{1.96^2 \ * \ 0.34(0.72)}{0.05^2}$$

n=376.16~377

Adding 10% to cater for non-response or missing values (0.1*377) +377=415

3.5 Sampling

This was a cross-sectional study carried out in April 2019 and all participants hailed from villages in East Pokot, Baringo County. Trachoma is Endemic in East

Pokot Google earth was used to map out a sampling area in the target location in East Pokot and selection was based on homesteads which are visible from google maps. The sub-locations were selected using Cluster random sampling. To achieve the most precise prevalence approximation, random sampling was done at both household and village levels for the reason that trachoma is known to cluster at the household and village level. The sample size was calculated as 400 people and all persons in selected households were sampled to get the sample size. To begin with, a region was selected in East Pokot and obtained from the county administrative office a list of all sub-locations in the selected study area. Cluster random sampling of 10 sub-locations was conducted and a simple random sampling within the clusters was done to select one village from each sub-location and a total of 10 villages were selected. Each village had community Health worker (CHW) who had a comprehensive list of all the households in their village and so using simple random sampling, 8 households per village were chosen at random from a hat. The sample size was 400 people, there is an average of 5 persons per household, a total of 10 villages were sampled and 8 households from each village were selected. The study team visited the selected homesteads, provided information on the study and then sought consent. Parents and guardians gave consent for the children to participate in study. Villagers who had received treatment for trachoma in the last two years and those who came from households outside the selection were excluded as they would have biased the study on prevalence. Also, MDA campaigns are accompanied with health education and behavior change.

3.6 Data collection Tools

A structured questionnaire (Appendix 1) and an observation check list (Appendix 2) was used to collect data. Prior to data collection, visits were made to mobilize

the community and to explain reasons and benefits for carrying out the study and benefits and any risks to the community.

3.6.1 Questionnaires

A structured questionnaire was used to collect social-demographic data for each household and information on factors predisposing the participants to trachoma. The head of household responded to the questionnaire. The questionnaires were translated into the local language and the researcher assisted the participants to fill in the questionnaires (Appendix 1).

Prior to the main study, a pre-test was undertaken in the neighboring Central Pokot which had similar characteristics to East Pokot but was not part of the study. The questionnaire was pre-tested among 10% of the study sample size to determine Validity and reliability. The questionnaire was supported by an observation check list (Appendix 2). The tool was found to be valid to test what it was intended and a reliable tool to consistently measure the study variables. Necessary corrections, adjustments and paraphrasing of questions was done to have a better comprehension of the tool.

The questionnaire was administered by field assistants who were drawn from the Fred Hollows eye clinic in Chemolingot sub-district hospital. All research assistants were trained on the ground on how to administer the questionnaire, interview and collect data prior to study commencing. Both the questionnaire and the consent form were translated into both Kiswahili and the local Pokot dialect. The research assistants who were both conversant in both Kiswahili and Pokot dialect explained information in the consent form (Appendix 3) and questionnaire and asked for their consent.

An observation checklist was used to collect data on the environmental hygiene and cleanliness within the compound of the households of the survey, latrine coverage and usage, distance to water sources, cleanliness of children faces, density of flies, water storage at the household level and the presence of cattle within the compound supported by an observation checklist (Appendix 2).

3.6.2 Standardized collection of eye swab

Eye swabs were taken using a sterile swab. The participant was asked to look upwards and the ophthalmologist gently parted the eyelids. The swab was placed on the inner part of the conjunctiva and gently rolled towards the outer part over the conjunctival sac inside the lower lid to collect epithelial cells. Gloves were changed between participants or at any time there was suspicion of contamination. The swab was placed back inside a covered lid. To check for contamination, three people were randomly selected at each village to receive an air swab. The swab was passed 5cm away without making contact with the participant. The samples were taken to the laboratory and PCR assays done to isolate the chlamydia pathogen. Participants who were picked out as having active trachoma (TF and/or trachomatous inflammation intense (TI) in one or both eyes) were supplied with two tubes of 1 % tetracycline eye ointment and taught how to apply it. Participants with trichiasis were referred for management by trained ophthalmologists.

3.7 Sample handling and storage

All samples were handled as hazardous material and on arrival at the laboratory stored at -30^{o}C.

3.8 Clinical diagnosis of trachoma

The diagnosis of trachoma was done clinically following WHO simplified trachoma grading for clinical diagnosis of trachoma namely based on inflammation of cornea; trachomatous inflation follicular (TF), trachomatous inflammation intense (TI), trachomatous scarring (TS), trachomatous trichiasis (TT) and corneal opacity-CO (WHO, 2003).

Table 3.1. Simplified grading scheme for assessment of trachoma in Communities

Grade	Clinical signs
Trachomatous inflammation follicular(TF)	The presence of five or more follicles of at least 0.5 mm Diameter in the central part of the upper tarsal conjunctiva.
Trachomatous inflammation, intense (TI)	Pronounced inflammatory thickening of the upper tarsal Conjunctiva obscuring more than half the normal deep tarsal vessels.
Trachomatous Conjunctival scaring (TS)	The presence of easily visible scars in the upper tarsal conjunctiva.
Trachomatous Trichiasis (TT)	At least one eye lash rubbing on the eyeball or evidence of recent removal of inward turned eyelashes.
Corneal opacity (CO)	Easily visible corneal opacity over the pupil so dense that at least part of the pupil is blurred when viewed through opacity.

3.9 Detection of Trachoma using PCR

DNA was extracted from eye swab and PCR (appendix 4) for *C. trachomatis* performed using the procedures described by Butcher, 2017. The DNA was extracted using TAKARA MightyAmp. *C. trachomatis C. psittaci* and *C. pneumonia DNA* was detected using primers of the OMP1 gene. The CT_F and

CT_R, Cps_F and Cps_R and Cpn-F and Cpn_R primers generate fragments that are 461bp, 355bp and 181bp. The amplification mixture contained 5.76ul water, 10ul buffer, 2ul additive, 0.84ul primer mix and 0.4ul polymerase. The mixture was denatured for 10secs at 98ºC. The samples were amplified in 40 cycles. Each cycle consisted of denaturation at 98ºC (10sec), annealing at 60ºC (15sec) and extension at 68ºC (35sec) (Schachter *et al*., 1973). The product was allowed to cool and stored at 4ºC. The amplification products were analyzed by electrophoresis in 2.5% agarose gel stained with 0.2% GelRed for 30 minutes. The gel was read using UV camera box (C1 program. ISO=400). A band of molecular weight corresponding to 461bp, 355bp and 181bp constituted of a positive result for *C. tracomati, C. psittaci* and *C. Pneumonia* respectively (Dean *et al*., 2008).

3.10 Data management and Analysis

Data was cleaned and validated by examining the questionnaire for completeness and consistency. All data was then entered into a compute spread sheet, Microsoft Excel and analyzed using XLSTAT. Descriptive statistics were used to summarize demographic profiles and determine prevalence expressed as mean (±SD), range, frequencies and percentages. Data was presented using graphs and frequency tables. Univariate and bivariate analysis using Chi-square for categorical variables. Multiple regression analysis to assess the independent effect of each determinant risk factor after controlling for all other factors and a T-test and Chi-square was used to compare means from the different populations. Statistical significance was set at $P<0.05$.

3.11 Ethical considerations

Permission to conduct the study was sought from Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Committee (Appendix 4). Written consent was further obtained from all study participants. The consent form (Appendix 3) was translated into Kiswahili, and the data collection personnel assisted the participants to read and understand before signing it. The local health and administration authorities were also notified of the study and their permission obtained.

CHAPTER FOUR

RESULTS

This was a cross sectional study carried out in East Pokot, Baringo County. The main objective of the study was to determine the prevalence, risk factors and molecular epidemiology of trachoma in East Pokot, Baringo ~~County~~.

4.1 Prevalence of trachoma among the Pokot people

4.1.1 Social-demographic and clinical characteristics of participants

Four hundred and five participants who met the inclusion criteria were enrolled in this study. Of those, 184 (45%) were male and 221 (55%) female. The age of the participants ranged from 3 days to 82 years, with a mean of 14.7 years and a median of 8 years. There were 204 (51%) children aged below nine years and 201 (49%) adults aged nine years and above. A total of 345 (85%) participants had no formal education, while 45 (11%) had primary education and 15 (4%) had secondary education. Whereas 196 (48%) of the participants were Christians, 209 (52%) practiced traditional religion. Of the homesteads, 342 (84%) were pastoralist, 32 (8%) were traders, 19 (5%) were charcoal burners, 8 (2%) were farmers, and 4 (1%) were teachers. A total of 367 (91%) participants used water from a river, 21 (15%) used public tap water, 7 (2%) used water from a protected public well and 10 (3%) used water from an open public well. For cooking fuel, 383 (95%) participants used charcoal while 22 (5%) used wood. A total of 369 (91%) participants lived in houses with earthen floors while 36 (9%) lived in houses with cemented floors. Three hundred and sixty (89%) participants lived in houses with thatched roofs, 31 (8%) in houses with corrugated iron roofs and 14

(4%) in houses with tin roofs. Three hundred and eighty (96%) participants used bush as a toilet facility, while 17 (4%) used owned a latrine in the homestead.

Table 4.1: Social-demographic and clinical characteristics of the participants

Characteristics					
Gender	Male 45% (184)	Female 55% (221)			
Age	> 9 years 51% (204)	≤ 9 years 49% (201)	Average 14.7years	Range 3days-82 years	Median 8 years
Educational level	No formal 85% (345)	Primary 11% (45)	Secondary 4% (15)		
Religion	Christianity 48% (196)	Ethnic 52% (209)			
Occupation	Pastoralist 84.44% (342)	Trader 7.9% (32)	Farmer 1.98% (8)	Teacher 0.99% (4)	Burning Charcoal 4.69% (19)
Source of drinking water	River 90.62 (367)	Public tap 5.19% (21)	Protected public well 1.73% (7)	Open public well 2.47% (10)	
Cooking fuel	Wood 5.43% (22)	Charcoal 94.57% (383)			
Floor type	Cement 8.89% (36)	Earth 91.11% (369)			
Roof Type	Thatch 88.89% (360)	Corrugated iron 7.65% (31)	Tin Roof 3.46% (14)		
Toilet facility	Bush 95.8% (388)	Own latrine 4.2% (17)			
Clinical diagnosis	Positive 44.44% (180)	Negative 55.56% (225)			
PCR results	Positive 14.07% (57)	Negative 89.93% (348)			

4.1.2 Prevalence of trachoma by age and sex among the Pokot people

A total of 405 samples were successfully screened for trachoma by the clinician using the WHO guidelines as described in figure 2.4 diagnosis of Trachoma. Of those, 225 (55.56%) screened negative while 180 (44.44%) screened positive for trachoma (Table 4.1).Ages 9 years and below were classified as children according to the WHO guidelines. Out of the 204 children aged below 9 years, 118 (57.8%) [95% CI: 1.04-1.81] had trachoma while out of the 201 adults aged 9 years and

above, 62 (34.3%) [95% CI: 0.33-0.60] had trachoma. The likelihood of having trachoma as a child was higher (odds: 1.37) compared to that of an adult (odds: 0.33). This was significant at P=0.000. Aggregated by gender, out of the 221 females, 97 (43.9%) [95% CI: 0.59-1.02] had trachoma whereas out of the 184 males, 101 (54.9%) [95% CI: 0.61-1.09] had trachoma. The likelihood of having trachoma as a male was higher (odds: 0.82) compared to that of a female (odds: 0.82). This was a however not a significant difference at P=0.81.

Table 4.2: Prevalence of trachoma among the Pokot people

Trachoma	**Frequency (n)**	**Percentage (%)**
Present	180	44.44
Absent	225	55.56
Total	**405**	

Table 4.3: Prevalence of trachoma by age and sex among the Pokot people

Variable	**Characteristic**	**Frequency (n)**	**Proportion with Trachoma**	**95% Confidence Interval**
Gender	Male	184	54.9% (101)	0.61-1.09
	Female	221	43.9% (97)	0.59-1.02
Age (years)	<9 years	204	57.8% (118)	1.04-1.81
	≥9 years	201	34.3% (62)	0.33-0.60

4.2 Circulating Chlamydia species

Conjunctiva swabs were taken successfully from all the 405 participants and shipped to the laboratory for polymerase chain reaction. There are different chlamydia species known to cause trachoma but tested three in this study. PCR was performed successfully on all the 405 eye swabs; 57 out of 405 (14.07%) samples were PCR positive. Twelve (12, 2.96%) of the samples tested positive for

Chlamydia trachomatis, 31 (7.65%) for *Chlamydia psittaci*, and 14 (3.46%) for *Chlamydia pneumonia*.

Table 4.4: Different Chlamydia species found using PCR

Chlamydia species	Absent	Present
Chlamydia trachomatis	97.04% (393)	2.96% (12)
Chlamydia psittaci	92.35% (374)	7.65% (31)
Chlamydia pneumoniae	96.54% (391)	3.46% (14)

4.2.1 PCR positive gel results

Agarose gel image in figure 4.2.1 shows an example of positive results of PCR amplification of chlamydia genomic DNA for clinical diagnosis. Bands show presence of different chlamydia species run on 2.5% agarose gel MW represents 100bp molecular weight. PC represents positive controls: *C. trachomatis* 461bp, *C. psittaci* 355bp and *C. pneumonia* 181bp and NC is the negative control. Lane 2 shows a positive band for *C. pneumonia*, lane 5 positive band for *C. psittaci* and lane 9 shows a positive band for *C. trachomatis* as shown in figure 4.2.1.

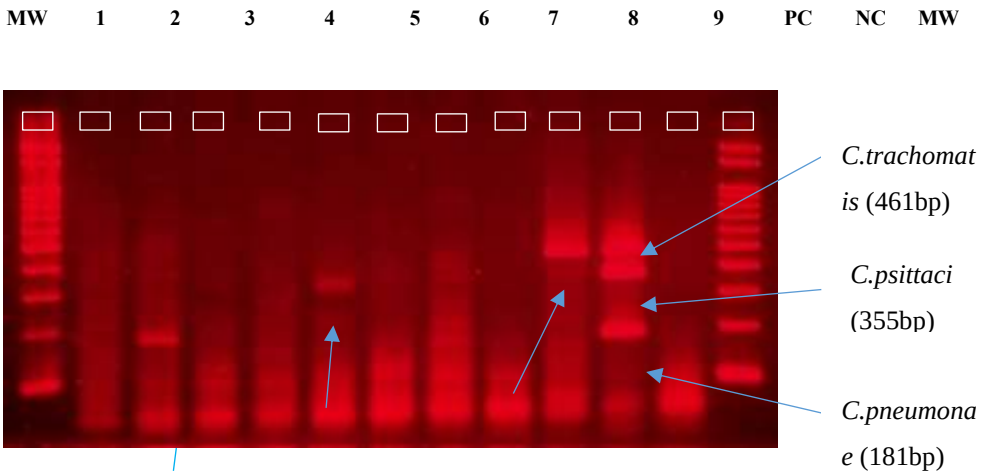

Figure 4.1: PCR positive gel results

4.2.2 Dual infection with different Chlamydia species

57 (14.07%) of the participants had co-infection with the three chlamydia species. None of the 405 study participants was infected with the three chlamydia species at once. 7 (1.73%) participants had dual infection with both *Chlamydia psitacci and Chlamydia trachomatis*, while 5 (1.23%) had dual infection *Chlamydia psitacci* and *Chlamydia pneumonia.* None of the participants had dual infection with *Chlamydia trachomatis* and *Chlamydia* pneumonia and none had triple infection. The dual infections are illustrated in figure 4.2.2

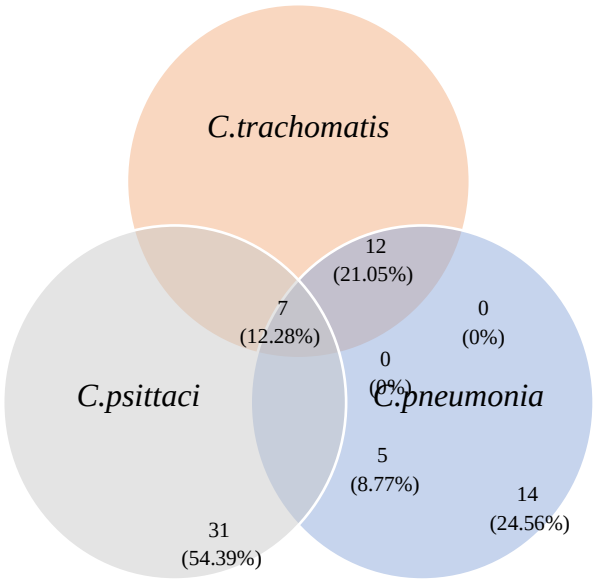

Figure 4.2: Dual infection with different chlamydia species

4.2.3 Agreement between clinical diagnosis and PCR results

A total of 180 (44.44%) out of the 405 participants were diagnosed as clinically chlamydia positive. Of these, 25 (13.89%) tested laboratory positive. On the other hand, 225 (55.56%) were diagnosed as clinically chlamydia negative and of these 20 (8.89%) tested chlamydia negative in the laboratory. The concordance was number positive from both test plus number negative from both tests, that is, (a+d)/(a+b+c+d)*100). Therefore, the overall concordance between the clinical diagnosis and laboratory diagnosis was therefore 29.94%.

Table 4.5: Agreement between clinical diagnosis and PCR results

		Trachoma		
		Present	**Absent**	**Total**
PCR	Positive	25 (13.89%)	205 (91.11%)	230 (56.79%)
	Negative	155 (86.11%)	20 (8.89%)	175 (43.21%)
	Total	**180 (44.44%)**	**225 (55.56%)**	**405 (100%)**

4.3 Factors Associated with Chlamydia positivity

Participants aged below nine years had significantly higher odds (OR 0.33, 95% CI 0.21- 0.49, p=0.000) of having trachoma infection when compared with those above nine years. Those who had primary and secondary education had significantly higher odds of having trachoma (OR 0.45, 95% CI 0.23-0.89, p=0.018) and (OR 0.28, 95% CI 0.08-1.01, p=0.037) infection than those who had no formal education. Gender was not significantly associated with positivity. The other factors that were significantly associated with trachoma positivity were; Using wood for cooking fuel (OR 3.83, 95% CI 1.26-11.64, P=0.011), having thatch roof (OR 6.31, 95% CI 2.12-18.82, P=0.0001), having an earthen floor (OR 4.46, 95% CI 1.79-11.14, P=0.0004), not having own latrine in compound (OR 0.16, 95% CI 0.03-0.71, P=0.0057), cattle rearing in the compound (OR 0.42, 95% CI 0.21-0.89, P=0.001), presence and high fly density (OR 0.28, 95% CI 0.10-0.52, P=0.000), having an unclean compound (OR 1.11, 95% CI 0.42-2.41, P=0.007), distance to water point that is greater than 2 hours (OR 0.35, 95% CI 0.15-0.66, P=0.004), religion (OR 0.97, 95% CI 0.0.65-1.42, P=0.008) and having an unclean face (OR 9.35, 95% CI 5.25-23.54, P=0.000). Occupation of head of household, source of drinking water were not significantly associated with positivity. The association between social demographic factors is presented in table 4.3a and 4.3b.

Table 4.6a: Factors associated with Chlamydia Positivity in children

		Trachoma			
Characteristic (n=263)	**Description**	**Frequency (%)**	**Odds Ratio**	**Confidence Interval**	**P Value**
Gender	Female	133 (50.6)	Ref	-	-
	Male	130 (49.4)	0.99	0.61-1.60	0.958
Education level	No formal school	230 (87.2)	Ref	-	-
	Primary	33 (12.6)	0.41	0.19 – 0.87	**0.021**
	Secondary	-	-	-	-
Occupation	Charcoal	12 (4.6)	ref	-	-
	Pastoralist	228 (86.7)	6.07	1.30 – 28.31	0.022
	Trader	13 (7.2)	4.50	0.77 – 26.29	0.095
	Farmer	2 (0.8)	-	-	-
	Teacher	2 (0.8)		-	-
Religion	Christian	119 (45.3)	Ref	-	-
	Ethnic	144 (54.8)	0.75	0.46 – 1.22	**0.258**
Water	Open public well	4 (1.5)	Ref	-	-
	Protected public well	3 (1.14)	-	-	-
	Public tap	11 (4.2)	0.67	0.04- 10.25	0.77
	River	245 (93.2)	3.68	0.38 – 35.90	0.26

Table 4.6b: Factors associated with Chlamydia Positivity in children

		Trachoma			
Characteristic (n=263)	**Description**	**Frequency (%)**	**Odds Ratio**	**Confidence Interval**	**P Value**
Cooking fuel	Charcoal	9 (3.4)	Ref	-	-
	Wood	254 (96.6)	4.03	0.82 – 19.79	**0.086**
Floor type	Cement		Ref	-	-
	Earth		4.34	1.18 – 15.94	**0.027**
Roof type	Corrugated iron	11 (4.2)	Ref	-	-
	Thatched	241 (91.6)	4.56	1.01 – 20.62	**0.049**
	Tin	11 (4.2)	-	-	-
Toilet facility	Bush	257 (97.7)	Ref	-	-
	Own latrine	6 (2.3)	0.18	0.02 – 1.52	**0.114**
Flies	Yes		Ref	-	-
	No		0.28	0.10-0.52	**0.000**
Cattle rearing	Yes		Ref	-	-
	No		0.42	0.21-0.89	**0.001**
Clean compound	Yes		Ref	-	-
	No		1.11	0.42-2.41	**0.007**
Distance to water point	< 2 hours		Ref	-	-
	≥ 2 hours		0.35	0.15-0.66	**0.004**
Clean face	Yes		Ref	-	-
	No		9.35	5.25-23.54	**0.000**

Factors associated with Chlamydia Positivity in adults

Characteristic (n=)	Description	Trachoma Frequency (%)	Odds Ratio	Confidence Interval	P Value
Gender	Female	88 (61.97)	Ref	-	-
	Male	54 (38.03)	0.87	0.41- 1.84	0.713
Education level	No formal school	115 (80.1)	Ref	-	-
	Primary	12(8.5)	0.42	0.09 – 2.02	**0.28**
	Secondary	15 (10.6)	0.53	0.14-1.98	**0.34**
Occupation	Charcoal	7 (4.9)	Ref	-	-
	Pastoralist	114 (80.3)	0.52	0.11-2.46	0.41
	Trader	19 (7.2)	0.83	0.13-5.40	0.848
	Farmer	6 (4.2)	0.67	0.07-6.41	0.725
	Teacher	2 (1.4)	-	-	-
Religion	Christian	77 (54.2)	Ref	-	-
	Ethnic	65 (45.8)	1.27	0.62-2.62	**0.5**
Water	Open public well	6 (4.2)	Ref	-	-
	Protected public well	4 (2.8)	0.67	0.04-11.3	0.78
	Public tap	10 (7.0)	0.5	0.05- 4.98	0.55
	River	122 (85.9)	0.87	0.15-4.96	0.42

Factors associated with Chlamydia Positivity in children

Characteristic (n=263)	Description	Trachoma Frequency (%)	Odds Ratio	Confidence Interval	P Value
Cooking fuel	Charcoal	13 (9.1)	Ref	-	-
	Wood	129 (90.9)	2.47	0.52-1.67	**0.253**
Floor type	Cement	22 (15.5)	Ref	-	-
	Earth	120 (84.5)	3.04	0.85-10.92	**0.087**
Roof type	Corrugated iron	20 (14.1)	Ref	-	-
	Thatched	119 (83.8)	4.56	1.01 – 20.62	**0.049**
	Tin	3 (2.1)	-	-	-
Toilet facility	Bush	131 (92.3)	Ref	-	-
	Own latrine	11 (7.8)	0.22	0.03-1.77	**0.155**
Flies	Yes		Ref	-	-
	No		0.28	0.10-0.52	**0.000**
Cattle rearing	Yes		Ref	-	-
	No		0.42	0.21-0.89	**0.001**
Clean compound	Yes		Ref	-	-
	No		1.11	0.42-2.41	**0.007**
Distance to water point	< 2 hours		Ref	-	-
	≥ 2 hours		0.35	0.15-0.66	**0.004**
Clean face	Yes		Ref	-	-
	No		9.35	5.25-23.54	**0.000**

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Prevalence of trachoma

In this study, prevalence of trachoma was found to be 44.44% using the WHO algorithm by a trained ophthalmologist. This prevalence is much higher than that reported in previous studies of 35.0% (Karimurio et al., 2006). Previous studies suggest that trachoma is endemic in Baringo and has been so for the last half century. This high prevalence can be attributed to the fact that the study included only rural populations where health facilities are scarce and sanitation is poor. Also, the WHO simplified grading system does not include all conjunctival features. These are some common features that may be mistaken for follicles such as, concretions (benign lipid deposits) and cobblestone papillae (fluid filled sacs). On PCR 14.07% (45/405) were confirmed to have active trachoma infection. This finding differs significantly from the clinical prevalence and suggests that the WHO algorithm has lower specificity. Regardless of the approach, trachoma is highly prevalent and a major cause of morbidity in East Pokot. Stratified by age and sex, children 9 years and below had higher odds of getting trachoma (OR=0.33, 95% CI 0.21-0.49, p=0.000) compared to adults. However, there was no significant difference between males and females, females had a lower prevalence than males and this was consistent with a study done in Narok (Karimurio *et al*., 2007) but in contrast to a study done in Tanzania (Zambrano *et al*., 2015). There was some evidence on the ground that AMREF, Queen Elizabeth Diamond Trust foundation and Ministry of Health were actively involved in

tackling trachoma and it was not clear to the study team why the prevalence would remain quite high.

5.1.1 Circulating trachoma species in East Pokot

Whereas previous studies determined the prevalence of *Chlamydia trachomatis*, this is the first study in East Pokot that describes the presence of *Chlamydia trachomatis*, *Chlamydia psittaci* and *Chlamydia pneumonia.* Of the 225 participants that were clinically diagnosed with trachoma, 57 (25.33%) were confirmed positive by PCR. On the other hand, of the 180 participants that the clinician determined to be trachoma free, 16 had trachoma by PCR. This means that the clinician could accurately detect trachoma in more than a fifth of those who actually had it, and detect the absence of trachoma in 91.11% of those who actually did not have trachoma. This suggests that the existing WHO guidelines used are more reliable at identifying non-cases and not nearly as good for detecting cases.

The study used PCR to identify the different chlamydia species circulating in East Pokot. Seven (7) participants had dual infection with both *Chlamydia psitacci and Chlamydia trachomatis,* while five (5) had dual infection *Chlamydia psitacci* and *Chlamydia pneumonia.* None of the participants had dual infection with *Chlamydia trachomatis* and *Chlamydia pneumonia* and none had triple infection and thus, this study found that multiple strains of chlamydiaceae are associated with trachoma. In Nepal a study reported the first evidence of involvement of multiple chlamydiaceae species in ocular trachoma. The bacteria they detected included *C. trachomatis*, *C. psittaci* and *C. pneumoniae* (Dean *et al*., 2008). A further 35% of the infected individuals had mixed infections which consisted

primarily of two chlamydiae species. Different species may be linked to severity and immune response to disease warranting novel approaches to the study and treatment as well as vaccines development for chlamydia.

5.1.2 Risk factors associated with trachoma

In this study, it was found that age had a significant effect on prevalence of trachoma with children having greater odds of disease. This is similar to other studies that indicate that children less than 9 years are more likely to be affected by chlamydia (West *et al*.,1991). Also, this study found that school going children had higher odds of having trachoma compared to those with no formal education. The reason for this is unclear but this study hypothesis is that older children attend school and are more likely to get infection from other school going children. A previous study conducted in a similar region with the same geographical and climatic conditions also found trachoma to be significantly higher among children less than 10 years compared to other ages (Karimurio *et al*., 2006). Children under 9 years are the main reservoirs of the bacteria for trachoma; children under this age cannot take care of themselves and thus are more affected as they are characterized by unclean faces, nasal discharge, food on the faces and dust which attract eye seeking flies which are the vector for trachoma. Being a Christian was associated with the decreased odds of having trachoma. It was noted that Christians tended to live near the town where there was running water and other amenities and the economic situation was better. Churches or mosques were only observed to be present in the town centers therefore, those who lived 30 minutes or more from the town center were more likely to practice an ethnic or traditional religion. The churches had amenities that were accessible to the congregants and also there were schools nearby so it is also possible that Christians had better

knowledge on hygiene practices and received informal education from the church. A study carried out in Tanzania in a similar setting found that those who practice Muslim religion had significantly lower odds of having trachoma infection (Zambrano *et al*., 2015). The Muslim faith requires its followers to frequently follow cleansing rituals so as to say their prayers which would mean they maintain a clean face. The findings of this study showed that those who had formal education had significantly higher odds of having trachoma infection. This is difficult to explain considering that formal education is expected to improve hygiene. On the other hand, one child with trachoma in a school can expose many others. Trachoma is highly contagious and would be easily spread in a school setting.

The general population has little access to health facility with the health center Chemolingot sub-county hospital been far to most population and therefore, chlamydia infection goes untreated. Several of the factors that are traditionally known to increase the likelihood of getting trachoma, including high domestic fly density, scarcity of water, open defecation and living close to domestic animals were observed. Indeed, the study team spent significant amounts of time devising means to avoid the clouds of flies perching on every exposed part of the body. The fact that so many are actively infected is of major concern, and blindness in old age is a distinct risk for many.

Although the study was not designed to identify blindness, it was noted that several instances in which blind adults were guided by young children, suggesting that many of those infected at an early age go on to become blind in later life. Clearly, access to clean water, prevention and treatment for Chlamydia infection should be made a priority by the County Health ministry

5.2 Study limitations

This study focused mainly on rural participants and did not collect data on urban dwellers and therefore could not generalize our findings to include the latter. Secondly, the study did not map households to infection due to resource limitation. This might have helped to identify households that are most affected. This study did not evaluate the epidemiological evidence concerning the extent to which climatic factors explain the current prevalence, distribution and severity of acute and chronic trachoma.

5.3 Conclusions

i. The clinical prevalence of trachoma was 44.44% while PCR test confirmed 14.07% to have active trachoma.
ii. The three species of trachoma; *Chlamydia trachomatis, Chlamydia psittaci* and *Chlamydia pneumonia,* were found to be in circulation in the study area. There was presence of dual infections but none of the study participants had triple infection.
iii. Adults and children who were 9 years and above, those practicing ethnic religion and having secondary level education were at higher odds of having trachoma infection.

5.4 Recommendations

- Trachoma is still a public health concern in East Pokot. The high prevalence is above the WHO target for elimination of trachoma in 2020. The prevalence was 44.44% contrary to WHO recommended threshold of 10%. This indicates a need for a rigorous health education and promotion

activities for trachoma awareness creation in the study area in order to control and prevent trachoma transmission and bring the prevalence levels down to below the <10% WHO threshold.

- There is need for county government of Baringo in collaboration with the National government to invest in accessible healthcare facilities as some communities have to travel for long distances to access the nearest sub-county hospital which is Chemolingot hospital.
- There is need for mass drug administration is important in attaining the WHO's goal of trachoma elimination by 2020 but they need to be cost effective in order to be a sustainable long term intervention tool. While administering MDA programs, it is important to assess whether transmission has decreased enough to stop treatment without resurgence of disease and in areas with low prevalence, this requires additional specific and sensitive surveillance tools.
- The addition of a sensitive and specific diagnostic tool would greatly enhance the ability of the clinicians to confirm their diagnosis and implement the right treatment and follow-up.
- Improvement in community and household sanitation such as provision of household latrines, help control fly breeding grounds and thus reducing their population. Increased access to water facilities, good hygiene practices is critical to achieving sustainable elimination of trachoma.
- Every effort must be made to provide treatment, and also routine prophylaxis. Furthermore, programs to supply clean water and to teach basic hygiene should be started and sustained. Open defecation ought to be discouraged. The pastoral nature of life in East Pokot could be replaced in some instances with sedentary agricultural activities.

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APPENDICES

Appendix I: Questionnaire

Interviewers name: Interview date:

Household questionnaire: (Household ID:)

1. Occupation of the household head:

☐ Farmer ☐ Teacher ☐ Office worker ☐ Mechanical/Factory worker
☐ Trader/market worker ☐ Fisherman ☐ Builder/construction worker ☐ Charcoal burning Others

2. What is your religion:

   1) Christian 2) Muslim 3) Hindu 4) Others 5) If others specify……..

3. Principal source of drinking water for your household:

1) Piped water in residence, 2) Piped water in compound, plot, 3) Piped water in public tap, 4) Open well in residence, yard, 5) Open public well, 6) Protected well in residence, yard, 7) Protected public well, 8) Springwater, 9) River, stream, pond, lake, dam, 10) Rainwater, 11) Bottled water, 12) other

4. Principal source of cooking fuel in your household;

1) Electricity, 2) LPG, natural gas, biogas, 3) Kerosene, 4) Charcoal, lignite, coal, 5) Wood, straw, dung, 6) Other

4. Principal type of toilet facility used by your household;

1) Private flush toilet, 2) Shared flush toilet, 3) Private pit latrine, 4) Shared pit latrine, 5) Private VIP latrine; 6) Shared VIP latrine, 7) Bush, field as latrine, 8) Other

5. What is the principal material used for the floors in your household?

1) Earth, mud, dung, sand; 2) Wood plank; 3) Parquet, polished wood; 4) Linoleum; 5) Tile; 6) Cement; 7) Carpet; 8) Other

6. What is the principal material used for the roof of your household?

1) Grass, thatch, makuti; 2) Tin cans; 3) Corrugated iron; 4) Asbestos sheets; 5) Concrete; 6) Tile; 7) Other

7. Is there presence of flies in the compound?

1) Yes 2) No

8. Do you rear cattle in the compound?

1) Yes 2) No

9. How long do you walk to and from to get domestic water?

1) Less than two hours 2) Two hours or more

10. How many times do you wash your face in a day?

1) Less than once 2) once or more

Individual questionnaire

ID: _____ name: _____ Blood sampling Number: __ __ __ __ __

Confirm age and sex: Age: __ __ Sex: _____

1. Education level :

No formal school, Primary school, secondary school, tertiary school

Graduated the above school? Yes, No

If currently go to school, give the name of the school

( )

Give the grade at school

( )

2. Any symptoms below?




Appendix II: Observation check list

Section A

Observation of the environment of the Manyatta. Yes No. Comment

1 Can a garbage bin/can be seen in the manyatta? **Yes/No**

2 Can a garbage can/bin be seen inside the manyatta? **Yes/No**

3 Is there a garbage hole for burning trash slightly

away from the manyatta? **Yes/No**

4 Is there a pit latrine in the compound or slightly

away from the manyatta? **Yes/No**

5 Does the latrine have a cover to block flies? **Yes/No**

6 Is there a water collection point near the manyatta? **Yes/No**

7 Is there a well? **Yes/No**

8 Is there a tap? **Yes/No**

9 Does the tap have running water? **Yes/No**

10 Is there a large tank for collecting water? **Yes/No**

11 Is there water in the tank? **Yes/No**

12 Is the tank covered to protect it? **Yes/No**

13 Are there food containers/utensils that are unwashed

and lying outside? **Yes/No**

14 Are the food containers/utensils covered to protect them from flies? **Yes/No**

Section B

Observation of the child (participant) Yes No. Comment

1 Is the face clean? **Yes/No**

2 Are there signs of dirt, flies, mucous secretions on eyes and nose? **Yes/No**

3 Are there signs of trachoma that can be seen?

(i.e. TF,TI,TS,TT,CO) **Yes/No**

Appendix III: Consent form-English version

Title of the study; Molecular Epidemiological study of Trachoma in East Pokot, Baringo County.

Investigators;

Joan Wangui- Jomo Kenyatta University of Science and Technology

Prof. Matilu Mwau - Kenya Medical Research Institute

Prof. Zipporah Ng'ang'a - Jomo Kenyatta University of Science and Technology

Study location;

East Pokot, Baringo County.

Consent forms for adults

Informed Consent for Adults aged 18 and above, in English.

My name is……and I work for the Ministry of Health. We are conducting a nationwide study on Neglected Tropical Diseases (NTDs). We think that Neglected Tropical Diseases are an important cause of illness in the community. The tests for some of these diseases are expensive and some others not commonly available. Our research team is trying to develop alternative tests that may detect diseases cheaply and may also detect diseases for which tests are not commonly available. We will use these tests to determine how important neglected diseases are in local communities. The information we gather is useful to the government and other policy makers who may consider preventative programs in this

community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including Ministry of Health, KEMRI, and others. The KEMRI's ethical review committee, who are responsible for conducting such reviews at national level, has approved this study.

Research Procedures: You are under no obligation to participate in this study that we are doing. If you agree to be a participant in this study, we will take a blood sample and then we will conduct some extra procedures as follows:

Blood sample: We will take a drop of blood through a finger prick. We will use sterile and disposable instruments that are clean and safe.

The extra samples taken from you will be transported to the KEMRI laboratories in Nairobi for analysis of Neglected Tropical Diseases. It may be necessary to transport your samples to a foreign laboratory for further testing using technology that is not readily available in the country. We are seeking permission from you to do this if it is necessary. It is possible that during this analysis, other infections may be found in your sample different from those commonly suspected. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but an identification number assigned to you will be used to label the sample.

Potential Risks: During this procedure there will be no long-lasting effect. However, you may feel a brief moment of pain or fear during the blood draw.

Potential benefits: This study may benefit your community since by helping the government and us to understand the problems your community is facing as a result of Neglected Tropical Diseases, we may be able to recommend and design

appropriate interventions to minimize the impact of these diseases. This study may benefit you because the results of the tests may be communicated to your doctor and to you.

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you. You can discuss with other members as you wish and you will be given a copy of the consent form to go with it.

Confidentiality: We will make every effort to protect your identity. You will not be identified in any report or publication of this study or its results.

Contact Information: If you have questions regarding this study you may contact the PI, Dr. Matilu Mwau on +254 728 073 633. If you have any question now or in the future regarding your rights or participation in this study, **you may contact the secretary, KEMRI Ethical Review Committee through Tel: +254 (020) 27222541, +254722205901, +254733400003 and Email: ERCAdmin@kemri.org**

Consent for the individual for blood sample:

May I now ask if you would like to participate in the study?

The above details about the study and the basis of participation have been explained to me and I agree to take part in the study. I understand that I am free to choose to be part of the study. I also understand that if I do not want to go on with the study, I can withdraw at any time. I give my consent for my blood to be

used for this study and can be shipped to a foreign country for further analysis if necessary. Please sign here or put your right hand thumb mark if you agree:

Signature/ Thumb mark----------

Date ----------

Witness Signature/ Thumb mark----------

Date ----------

Assent for minors

We are conducting the study in the whole of Kenya. We think that Neglected Tropical Diseases are an important cause of illness in the community. The tests for some of these diseases are expensive and some others not commonly available. Our research team is trying to develop alternative tests that may detect diseases cheaply and may also detect diseases for which tests are not commonly available. We will use these tests to determine how important neglected diseases are in local communities. The information we gather is useful to the government and other policy makers who may consider preventative programs in this community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including Ministry of Health, KEMRI, and others. The KEMRI's ethical review committee, who are responsible for conducting such reviews at national level, has approved this study.

Research Procedures: Your child is here to have a little blood drawn for some tests. If you allow your child to participate in this study we will take a drop of blood from the figure then we will conduct some extra procedures as follows:

Blood sample: We will take a drop of blood (finger prick) during the procedure. We will use sterile and disposable instruments that are clean and safe.

The extra samples taken from you will be transported to the KEMRI laboratories in Nairobi for analysis of Neglected Tropical Diseases. It may be necessary to transport your samples to a foreign laboratory for further testing using technology that is not readily available in the country. We are seeking permission from you to do this if it is necessary. It is possible that during this analysis, other infections may be found in your sample different from those commonly suspected. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but an identification number assigned to you will be used to label the sample.

Potential Risks: During this procedure there will be no long-lasting effect. However, your child may feel a brief moment of pain or fear during the blood draw.

Potential benefits: This study may benefit your community since by helping the government and us to understand the problems your community is facing as a result of Neglected Tropical Diseases, we may be able to recommend and design appropriate interventions to minimize the impact of these diseases. This study may benefit your child because the results of the tests may be communicated to your child's clinician and to you.

Participant's Rights: Your child's participation in this study is voluntary and if you or the child declines to participate, y services that are normally available will not be denied. You can discuss with other members as you wish your child will also sign an assent form. We will make every effort to protect your child's identity.

Confidentiality: We will make every effort to protect your child's identity. Your child will not be identified in any report or publication of this study or its results.

Contact Information: If you have questions regarding this study you may contact the PI, Dr. Matilu Mwau on +254 728 073 633. If you have any question now or in the future regarding your rights or participation in this study, **you may contact the secretary, KEMRI Ethical Review Committee through Tel: +254 (020) 27222541, +254722205901, +254733400003 and Email: ERCAdmin@kemri.org**

Consent for the individual for blood sample: May I now ask if you will allow your child to participate in this study?

The above details about the study and the basis of participation have been explained to me and I allow my child to take part in the study. I understand that I am free to allow my child to be part of this study. I also understand that if I do not want him/her to go on with the study, I can withdraw at any time. I give my consent for my child's blood to be tested for Neglected Tropical Diseases and can be shipped to a foreign country for further analysis if necessary. Please sign here or put your right hand thumb mark if you agree:

Signature/ Thumb mark----------

Date ----------

Appendix IV: PCR protocol

1. Add the following components to a sterile 0.5 ml microcentrifuge tube sitting on ice:

	f/c	X1 (uL)	X26
Water		5.76	149.76
2x Buffer V3	1x	10.0	260.0
10x additive	1x	2.0	52.0
Chlamydia 4-multiplex primer mix	0.3uM or 0.15uM	0.84	21.84
MightyAmp V3 polymerase(1.25U/uL)	1.25U/50uL	0.4	10.4
Trachoma lysate		1.0	unique
total		20.0	494.0

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube. (Add 50 µl of mineral oil to the top of each tube to prevent evaporation if using a thermal cycler without a heated lid).
3. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template. Perform 25–35 cycles of PCR amplification as follows: Denature 94°C for 45 s Anneal 55°C for 30 s Extend 72°C for 1 min 30 s
4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at –20°C until use.
5. Analyze the amplification products by electrophoresis gel and visualize by fluorescent nucleic acid stain. Use appropriate molecular weight standards.