

Genetic Diversity of *Cryptosporidium Spp.* in Njoro Sub County, Nakuru, Kenya

Walter Miding'a Essendi (✉ walter.essendi@egerton.ac.ke)

Egerton University <https://orcid.org/0000-0003-0989-0030>

Charles Inyagwa Muleke

Egerton University - Njoro Campus: Egerton University

Miheso Manfred

KENYA AGRICULTURAL AND LIVESTOCK RESEARCH INSTITUTE

Elick Onyango Otachi

Egerton University - Njoro Campus: Egerton University

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Abstract

Cryptosporidium spp. cause Cryptosporidiosis in humans through zoonotic and anthroponotic transmission. Previous studies have illustrated the significance of domestic animals as reservoirs of this parasite. However, there is no information on the *Cryptosporidium spp.* and genotypes circulating in Njoro Sub County. A total of 2174 samples from humans, cattle, chicken, sheep and goats were assessed for presence of *Cryptosporidium spp.* Thirty-three positive samples were successfully sequenced. The sequences obtained were compared to *Cryptosporidium* sequences in the GenBank using NCBI's (National Center for Biotechnology Information) online BLAST (Basic Local Alignment Search Tool) algorithmic program. Sequence alignment was done using the Clustal W program and phylogenetic analysis was executed in MEGA 6 (Molecular Evolutionary Genetics Analysis version 6.0). The *Cryptosporidium spp.* present in the watershed showed great genetic diversity with nine (9) *Cryptosporidium spp.* namely: *C. parvum*, *C. hominis*, *C. ubiquitum*, *C. meleagridis*, *C. andersoni*, *C. baileyi*, *C. muris*, *C. xiaoi* and *C. viatorum*. Cattle were the biggest reservoirs of zoonotic *Cryptosporidium spp.* hence a potential source of zoonosis in humans while goats had the least species. This is the first study that reported presence of *C. viatorum* in Kenya.

Introduction

Cryptosporidium is a common zoonotic parasite responsible for global outbreaks of waterborne gastrointestinal diarrhea (Zahedi et al. 2016). Cryptosporidiosis contributes to a high number of unreported child morbidity and mortality, especially in Africa (Shirley et al. 2012). However, the accurate global load of this disease remains a mystery not only due to lack of a simple and affordable diagnostic tool but also due to unacknowledged frequency and severity of the disease in immunocompetent patients (Shirley et al. 2012). Recent studies have demonstrated that anthroponotic transmission of *Cryptosporidium* predominates over zoonotic transmission in regions with poor hygiene (King et al. 2019). Domestic animals such as cattle, goats and sheep are vital sources of zoonotic *Cryptosporidium spp.* (Walter et al. 2021). However, there are other animals, such as poultry and rabbits, which happen to be important sources of emerging zoonotic species, for instance *C. meleagridis* and *C. cuniculus* respectively (Robertson et al. 2020). This highlights the significance of evaluating livestock production systems in Africa for their potential in zoonotic transmission of cryptosporidiosis.

Although Putignani and Menichella (2010) documented *Cryptosporidium parvum* as the species that causes most zoonotic infections in developed countries and slums in developing countries, other species circulate in farm animals, rodents and humans in rural areas (Robertson et al. 2020). Therefore, immense genetic diversity occurs among *Cryptosporidium spp.* in various hosts and geographical areas (Feng et al. 2018). This survey focused on the identification of the genetic characteristics of the *Cryptosporidium spp.* circulating in a rural setting, Njoro Sub County, in Kenya.

River Njoro is a source of drinking water for both humans and domestic animals within the Njoro river watershed. The river could be polluted by waterborne pathogens shed by domestic animals, which can then infect humans (Jenkins and Maina-Gichaba 2009). The prevalence and distribution of zoonotic species of *Cryptosporidium* in Njoro Sub County is a public health concern. Therefore, the aim of this study was to

determine the prevalence and genetic diversity of *Cryptosporidium* isolates in humans and domestic animals in Njoro Sub County.

Materials And Methods

Study area

The study was conducted in River Njoro watershed (Fig.1), in Njoro Sub County in Nakuru County, Kenya, which lies between longitudes 35°05'E and 36°05'E, and latitudes 0°15'S and 0°25'S (Mainuri and Owino 2013). River Njoro has its origin in the Eastern Mau, covers about 50km in length and an estimated surface area of about 270km². River Njoro watershed comprises of forested, agricultural lands and urban settlements and terminates in Lake Nakuru (Mainuri and Owino 2013)

Study design

The study employed a Complete Randomized Design to ensure each region sampled within the Njoro Sub County had an equal chance of being included. Purposive sampling design was utilized when sampling humans, where only patients who showed signs of diarrhea were recruited as study participants due to their higher probability of cryptosporidiosis infection. Hospitals and homesteads were randomly selected. Another inclusion criterion was based on consideration of individuals who resided within Njoro Sub County during the entire study period.

Samples and sample collection

The units of sampling were humans and domestic animals such as cattle, sheep, goats and chicken while the samples used in the study were human and animal fecal matter. A total of 2174 fecal samples were randomly collected, each weighing 10g, and distributed as follows: 378 from human, 1000 cows, 388 sheep, 88 from goats and 320 from chicken. The animal owners were interviewed on the source of water given to the animals. The samples collected were transported to Egerton University, Biological Science laboratory and preserved at 4°C.

Identification of *Cryptosporidium spp.* in stool samples

All the fecal samples were analyzed for the presence of *Cryptosporidium spp.* oocysts using the Ziehl – Neelsen staining technique as described by Henriksen and Pohlenz (1981). Smear slides were air dried and then examined under the microscope at ×40 magnification. *Cryptosporidium spp.* oocysts appear as pink to red, spherical to ovoid bodies against a green to purple background. Samples were considered positive if at least one morphologically distinct *Cryptosporidium spp.* oocyst was observed.

DNA extraction, Quantitation and PCR

DNA extraction was done using the Zymo gDNA extraction kit following the manufacturer's protocol with modifications. Modifications included; increasing the lysis temperature to 80°C to enhance denaturation of nucleases, deactivation of PCR (Polymerase Chain Reaction) inhibitors and lysis of

cryptosporidium cysts. Initial centrifugation time was increased from 5 minutes to 10 minutes to increase efficiency of separation. Additionally, the incubation time was increased to 6 hours to increase DNA precipitation.

DNA recovery was measured using Nano drop spectrophotometer and agarose gel electrophoresis. Gel electrophoresis involved comparison of the intensity of ethidium bromide-stained DNA bands on agarose gels and Lambda DNA of molecular weight 50ng.

Inqaba Biotech Limited (Pretoria, South Africa) synthesized primers designed by Kim et al. (1992) on contract. PCR reactions were carried out in Eppendorf 2700 thermal cycler. PCR involved amplification of the full-length SSU rRNA gene by conventional PCR by using outer primer (forward 5'-AACCTGGTTGATCCTGCCAGTAGTC-3' and reverse primer 5'-TGATCCTTCTGCAGGTTACCTACG-3') and inner primers: (forward: 5'-GGAAGGGTTGTATTTATTAGATAAAG-3' reverse: 5'-AAGGAGTAAGGAACAACCTCCA-3'). The following PCR profile was used; 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 60 s; an initial denaturation incubation at 94°C for 5 min and a final extension incubation at 72°C for 10 min.

DNA sequencing

DNA amplicons were sequenced on contract by Inqaba Biotech Limited (Pretoria, South Africa), using Sanger sequencing platform. The sequencing raw data (chromatographs) were subjected to format conversion, quality control, trimming and filtering processes, all executed in Bioedit. The edited sequences were compared to *Cryptosporidium* sequences in the GenBank using NCBI's online BLAST tool with the default algorithm parameters to target 100 sequences. Sequence identity was characterized in terms of percentage identity, query coverage, Maximum Score, Total Score and E value.

Phylogenetic analysis

The sequences were aligned using the Clustal W program (Xiao et al. 2001) with manual adjustment. Phylogenetic analysis involved construction of a neighbor-joining (NJ) tree (Spano et al. 1997). The reliability of the NJ tree was assessed by the bootstrap method (Santos et al. 2007) with 1,000 replications. Statistically significant value was set at 95% (Babaei et al. 2011); however, values greater than 70% are reported because bootstrap values have been known to conservatively estimate the reliability of clades (Pedraza-Diaz et al. 2001). NJ and bootstrap analyses were performed using the MEGA 6 (Gonin and Trudel 2003). The relative distances among different *Cryptosporidium* spp. were calculated using the Kimura two-parameter method. In order to assess the relatedness of the genus *Cryptosporidium* with other members of the phylum Apicomplexa, *Babesia microti* (GenBank accession no. U53448) sequence was used as the outgroup. *B. microti* was used as the outgroup because it has been reported to be the most divergent member of this group.

Results

Prevalence of *Cryptosporidium* in Njoro Sub County

A total of 2174 samples from chicken, cattle, humans, sheep and goats were assessed for presence of *Cryptosporidium spp.* in Njoro Sub County; 6.99% (152/2174) of which were positive. Of the positive samples; 25/320 (7.81%) were from chicken, 108/1000 (10.8%) from cattle, 37/378 (9.8%) humans, 76/388 (19.6%) sheep and 4/88 (4.5%) goats (Table 1). Based on the results, *Cryptosporidium* infection was highest in cattle (10.8%) and lowest in goats (4.5%). The prevalence of Cryptosporidiosis in humans during the study period was 9.8%.

DNA extraction and PCR

Gel electrophoresis comparing the intensity of ethidium bromide-stained DNA bands on agarose gels and Lambda DNA of molecular weight 50ng appeared as shown in Fig 2.

Genotyping of *Cryptosporidium* isolates in Njoro Sub County

A total of 33 samples were successfully sequenced; 11 from chicken, 8 from cattle, 6 from humans, 5 from sheep and 3 from goats. In the study, nine *Cryptosporidium* species were identified from all the sources evaluated; human, cattle, sheep, goats and chickens. *C. parvum* was the predominant species having been detected in 27.27% (9/33) of the samples evaluated and from four sources; cattle, humans, chickens and sheep. *C. hominis* was detected in 15.15% (5/30) and humans were the only reservoir for this species. *C. ubiquitum* was identified in 18.18% (6/33) of the samples and it was detected in four sources; humans, sheep, goats and chicken. *C. meleagridis* was detected in 12.12% (4/33) samples exclusively from chicken. *C. andersoni* was identified in 12.12% (4/33) of the samples evaluated and in chicken and humans. *C. baileyi*, *C. muris*, *C. Xiao* and *C. viatorum* were each identified in only one sample and from a single source. The results of the 18 rRNA gene sequences of *Cryptosporidium spp.* in humans and animals in Njoro Sub County was summarized in table 2.

Proportion of *Cryptosporidium spp.* in domestic livestock

Evaluation of animal sources revealed that cattle were the highest reservoirs of *Cryptosporidium spp.* having four different species (*C. andersoni*, *C. bovis*, *C. muris* and *C. parvum*), two of which were identified as being zoonotic (*C. andersoni*, and *C. parvum*). Three species were detected in chicken (*C. andersoni*, *C. meleagridis* and *C. parvum*) all of which documented as capable of zoonotic transmission. Goats harbored three species of *Cryptosporidium* (*C. baileyi*, *C. ubiquitum*, and *C. xiao*) only one of which was identified as being capable of zoonotic transmission (*C. ubiquitum*). Sheep were identified to be the source of two species; *C. ubiquitum* and *C. parvum*, both of which are capable of being transmitted from animals to humans (Table 3). Three species were detected to be circulating in humans in Njoro Sub County (*C. hominis*, *C. andersoni* and *C. parvum*).

Zoonotic *Cryptosporidium spp.* circulating in Njoro Sub County

Maximum likelihood analysis of the sequences grouped the 33 isolates into two evolutionary clusters and three sub clusters (Fig. 2). Clustering was random and no significant host clustering among the 33 isolates was observed (Fig. 2).

Discussion

The prevalence of *Cryptosporidium* spp. reported in this study (6.99%) is consistent with results from other studies, which demonstrated its prevalence in Africa to occur in the range of 3-20% (Current and Garcia 1991). Our study show that *Cryptosporidium* is one of the most common gastrointestinal pathogen in humans, with a prevalence of 9.8%, almost similar to the 9% infection rates reported among children in Tanzania (Cegielski et al. 1999). However, higher infection rates of cryptosporidiosis have been reported in other studies, which include 17% in Egypt (Abdel-Messih et al. 2005) and 32% among children in Guatemala (Laubach et al. 2004).

This study identified nine species of *Cryptosporidium* through sequencing. These species include; *C. parvum*, *C. hominis*, *C. andersoni*, *C. ubiquitum*, *C. meleagridis*, *C. bovis*, *C. muris*, *C. viatorum* and *C. xiaoi*. In our study, majority of human infections were caused by *C. hominis* and the cattle genotype, *C. parvum*. These results are in agreement with those of a documented genotypic survey on the prevalence of Cryptosporidiosis among children with persistent diarrhea at Mulago Hospital in Uganda (Tumwine et al. 2003). The afore-mentioned Ugandan study demonstrated that 73.7% of the infections were caused by *C. hominis*, 18.4% were due to *C. parvum* while 3.9% were mixed infections with both species. Elsewhere, an epidemiologic study of cryptosporidiosis among children in Malawi illustrated that out of 43 cases, *C. hominis* was responsible for two with *C. parvum* (Morse et al. 2007) causing 41 cases. Because *C. hominis* and the cattle genotype *C. parvum* cause majority of human infections, the sources of these species are the main reservoirs of human cryptosporidiosis (Razakandrainibe et al. 2018). While humans are the only notable source of *C. hominis*, both humans and ruminants are the principal sources of the cattle genotype, *C. parvum* (Xiao et al. 2001). The cattle genotype of *C. parvum* has been found in other mammals. However, infected humans, cattle and sheep shed high numbers of oocysts, especially when infected during infancy; this probably poses a major risk to the environmental contamination (Putignani and Menichella 2010).

According to Pumipuntu and Piratae (2018), transmission of cryptosporidiosis occurs through direct or indirect contact with stools of animals. Outbreaks occur through various routes of transmission: person-to person contact in institutions, animal contact during farm visits, and contact with recreational waters, swimming pools, municipal drinking water and food (Chalmers 2012). Previous studies identified human-to-human contact as the most common means of transmission (Cordell and Addiss 1994). This is illustrated by the increased risk of outbreaks in areas where there is routine crowding, such as day-care centers and schools, or patient-patient and patient-staff transmission in hospitals and the ultimate spread to the family members of the attending children or staff (Casemore 1990; Cordell and Addiss 1994). *C. parvum* is the most documented *Cryptosporidium* spp. involved in zoonotic transmission (Zahedi et al. 2016). Most of the reported cases of outbreaks of cryptosporidiosis in schoolchildren after exposure to calves or lambs are because of *C. parvum* (Casemore 1990; Casemore et al. 1997). *C. parvum* cryptosporidiosis has also been implicated in infection resulting from occupational exposure to infected animals (Current, 1994; Casemore et al. 1997). Furthermore, evidence from genetic analysis has proven that only the cattle genotype of *C. parvum* is capable of zoonotic transmission (Sulaiman et al. 1999). However, this genotype has also been found in many other host species such as humans, cattle, pigs and sheep (Putignani and Menichella 2010).

The high prevalence of the *C. parvum* in cattle and sheep coupled with the high numbers of oocysts shed by infected animals, especially newborns, make cattle and sheep important sources of environmental pollution with *Cryptosporidium* oocysts, which are capable of infecting humans (Uga et al. 2000).

The present study also detected *C. parvum* in sheep and chickens. This is in contrast with many past studies which had demonstrated a rare occurrence of *C. parvum* in small ruminants and birds in Africa (Robertson et al. 2020); with *C. xiaoi* predominating in sheep and goats in Ghana (Squire et al. 2017) and *C. ubiquitum* predominating in lambs under 5 years in Ethiopia (Wegayehu et al. 2017).

Although *C. ubiquitum* has a zoonotic potential, human infections in Africa have only been detected on rare occasions (Li et al. 2014). The present study established the existence of this species in goats, sheep and chickens. However, since these animals are likely to ingest *C. ubiquitum* oocysts in feces of infected children while feeding on grass, zoonotic transmission of this species is feasible in this case (Pumipuntu and Piratae 2018).

This study identified *C. andersoni* sp. in some of the chicken and human samples analyzed. Recent studies have demonstrated the emergence of *C. andersoni* as a major species causing cattle cryptosporidiosis, after *C. parvum* especially in calves (Wang et al. 2019). *C. andersoni* has also been detected in other animal species, such as cattle, sheep, horses, camels, and ostriches (Liu et al. 2020). In this study, the human *C. andersoni* was identical to the SSU rRNA gene of two *C. andersoni* isolates derived from chicken and cattle (XVaA3h and XVaA3g). The same subtypes have been identified in humans (Braima et al. 2019; Xu et al. 2020) and rats (Chen et al. 2019). Results reported in the study indicated that *C. andersoni* has a significant zoonotic potential.

The present study discovered a novel isolate of *Cryptosporidium viatorum* in human in Njoro Sub County, Kenya. This therefore increases the number of countries in which *C. viatorum* has been detected to 10: "Australia (n = 1) (Braima et al. 2019), China (n = 1) (Xu et al. 2020), Colombia (n = 1) (Sánchez et al. 2017), Ethiopia (n = 12) (Adamu et al. 2014; Stensvold et al. 2015; de Lucio et al. 2016), India (n = 2) (Khalil et al. 2017; Khalil et al. 2018), Myanmar (n = 1), Nigeria (n = 2) (Ayinmode et al. 2014; Ukwah et al. 2017), Sweden (n = 3) (Insulander et al. 2013; Stensvold et al. 2015), and the UK (n = 14) (Elwin et al. 2012; Stensvold et al. 2015)," and presently Kenya (n = 1). Currently, the source of infection of *C. viatorum* in Njoro Sub County is unknown. *C. viatorum* was initially thought to occur exclusively in humans. However, its detection has also been made in some rat species in Australia (Koehler et al. 2018) and China (Chen et al. 2019; Zhao et al. 2019).

Several studies in Africa have reported the presence of *C. meleagridis* infections in both immunocompromised and non-immunocompromised individuals, especially children (Hunter and Nichols 2002; Robertson et al. 2020). In this study, chicken were the sources of *C. meleagridis* and because this species has been identified as a zoonotic *Cryptosporidium* sp, (Zahedi et al. 2016), chickens may be a potential reservoir. This observation is similar with findings from Côte d'Ivoire (Berrilli et al. 2012) and Nigeria (Ayinmode et al. 2018) which suggested that there existed an association with chicken but in contrast with numerous other studies from Africa which did not indicate any association with infected animals or birds (Mbae et al. 2015) and instead emphasized human to human transmission.

This study identified *C. baileyi*, *C. muris*, and *C. xiaoi*, each from a single source. Species such as *C. muris* and *C. xiaoi* have been previously detected and identified in immunocompromised individuals (Chappell et al. 2015); while *C. bovis*, and *C. muris* have been detected in immunocompetent humans, especially children (Azami et al. 2007).

Conclusions And Recommendations

This study confirmed that cryptosporidiosis is prevalent in Njoro Sub County and domestic animals are important reservoirs and a potential source of zoonosis in humans. The *Cryptosporidium spp.* present in the Njoro river watershed show great genetic diversity with *C. viatorum* having been detected for the first time in Kenya. Extensive epidemiological and genomic studies of animal reservoirs of *C. viatorum* in Kenya are therefore required in order to clarify whether the transmission of this species is zoonotic or anthroponotic.

Declarations

Funding

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Conflict of Interest/Competing interest

The authors declare that there is no conflict of interest and have no competing interest in this study.

Availability of data and material

The datasets that support the findings of the current study have been deposited in a repository, GenBank, awaiting publication and accession numbers.

Code availability

Not applicable

Authors' contributions

Walter Miding'a Essendi, Charles Muleke and Elick Otachi contributed to the study conception and design. Material preparation, data collection and analysis were performed by Walter Miding'a Essendi and Manfred Miheso. The first draft of the manuscript was written by Walter Miding'a Essendi and all authors critically reviewed all the versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This research was done upon approval by research and ethical committee of Egerton University (EUREC/APP/093/2019; Date: 4/3/2020).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable

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Tables

Table 1: Summary of prevalence of Cryptosporidiosis in Njoro Sub County

Host	Total number of samples collected	n/(%) samples Positive for <i>Cryptosporidium</i>	Number of Positive Samples Successfully Genotyped
Birds	320	25 (7.81%)	11
Cattle	1000	108 (10.8%)	8
Humans	378	37 (9.8%)	6
Sheep	388	76 (19.6%)	5
Goats	88	4 (4.5%)	3
Total	2174	152 (6.99%)	33

Table 2: Comparison of the 18S rRNA gene sequences of *Cryptosporidium* from domestic animals and humans in Njoro Sub County to sequences of *Cryptosporidium* in the GenBank by BLAST analysis (updated by 20 Feb, 2021)

Code	Origin	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
C31	Cattle	<i>Cryptosporidium andersoni</i> isolate SMX467154	797	797	100%	0	100.00%	MN379941.1
C88	Cattle	<i>Cryptosporidium bovis</i> isolate Y6	763	763	100%	0	99.06%	MF348255.1
C15	Cattle	<i>Cryptosporidium muris</i> isolate M1093-5649	1454	1454	100%	0	100.00%	KJ469983.1
C60	Cattle	<i>Cryptosporidium parvum</i>	1633	1633	100%	0	96.11%	DQ389176.1
C30	Cattle	<i>Cryptosporidium parvum</i>	1633	1633	100%	0	96.11%	DQ389176.1
C24	Cattle	<i>Cryptosporidium parvum</i> isolate 8934	1443	1443	97%	0	99.01%	DQ648547.1
C42	Cattle	<i>Cryptosporidium parvum</i> isolate IQ.No.10	952	952	100%	0	100.00%	MT645530.1
C71	Cattle	<i>Cryptosporidium parvum</i> isolate SY90	1408	1408	100%	0	98.98%	MT374186.1
X512	Chicken	<i>Cryptosporidium andersoni</i> isolate SMX467154	1520	1520	100%	0	100.00%	MN379941.1
XC14	Chicken	<i>Cryptosporidium andersoni</i> isolate ZYNC2	NA	117008	401	1.00E-111	100.00%	MT636368.1
X511	Chicken	<i>Cryptosporidium meleagridis</i> isolate CryZaBr98	1291	1291	100%	0	100.00%	MF405462.1
X56	Chicken	<i>Cryptosporidium meleagridis</i> isolate CryZaBr98	1291	1291	100%	0	100.00%	MF405462.1
X514	Chicken	<i>Cryptosporidium meleagridis</i> isolate CryZaBr98	1291	1291	100%	0	100.00%	MF405462.1
XC18	Chicken	<i>Cryptosporidium meleagridis</i>	1291	1291	100%	0	100.00%	MF405462.1

		<i>isolate CryZaBr98</i>							
X59	Chicken	<i>Cryptosporidium parvum isolate BZ17A3</i>	1219	1219	100%	0	97.49%	MN557141.1	
X513	Chicken	<i>Cryptosporidium parvum isolate IQ.No.10</i>	952	952	100%	0	100.00%	MT645530.1	
X56	Chicken	<i>Cryptosporidium parvum isolate QH2</i>	819	819	100%	0	100.00%	KY859697.1	
X58	Chicken	<i>Cryptosporidium ubiquitum isolate Shivamogga</i>	NA	857276	944	0	100%	MT013495.1	
G7	Goat	<i>Cryptosporidium baileyi isolate IQ.No.14</i>	957	957	100%	0	100.00%	MT645534.1	
G10	Goat	<i>Cryptosporidium ubiquitum isolate Shivamogga</i>	1291	857276	944	0	100%	MT013495.1	
G6	Goat	<i>Cryptosporidium xiaoi isolate AH S1</i>	929	929	100%	0	100.00%	MH049731.1	
H19	Human	<i>Cryptosporidium andersoni isolate SMX467154</i>	1520	1520	100%	0	100.00%	MN379941.1	
H18	Human	<i>Cryptosporidium hominis isolate AW112</i>	608	608	100%	7.00E-170	100.00%	KC679688.1	
H12	Human	<i>Cryptosporidium hominis isolate ET91</i>	1452	1452	100%	0	100.00%	MK990042.1	
H23	Human	<i>Cryptosporidium hominis isolate Human-IQ10</i>	351	351	100%	9.00E-97	98.98%	MK886609.1	
H24	Human	<i>Cryptosporidium hominis isolate Human-IQ5</i>	1062	1062	100%	0	100.00%	MK886604.1	
H21	Human	<i>Cryptosporidium hominis isolate ZP-174</i>	1068	1068	100%	0	100.00%	MK982514.1	
H08	Human	<i>Cryptosporidium viatorum isolate SSU-rRNAIS20</i>	905	905	100%	0	100.00%	KX174309.1	

S5	Sheep	<i>Cryptosporidium parvum</i> isolate QH2	819	819	100%	0	100.00%	KY859697.1
S3	Sheep	<i>Cryptosporidium ubiquitum</i> isolate 14668	1528	1528	100%	0	95.59%	MF411077.1
S2	Sheep	<i>Cryptosporidium ubiquitum</i> isolate 14668	1528	1528	100%	0	95.59%	MF411077.1
S9	Sheep	<i>Cryptosporidium ubiquitum</i> isolate 14668	1528	1528	100%	0	95.59%	MF411077.1
S12	Sheep	<i>Cryptosporidium ubiquitum</i> isolate 14668	1572	1572	100%	0	100.00%	MK573335.1

Table 3: Proportion of *Cryptosporidium* spp. in domestic livestock

Source	Total Number of <i>Cryptosporidium</i> species	Zoonotic <i>Cryptosporidium</i> species
Human	3	2 (66.67%)
Cattle	4	2 (50%)
Chicken	3	3 (100%)
Goats	3	1 (33.33%)
Sheep	2	1 (50%)

Figures

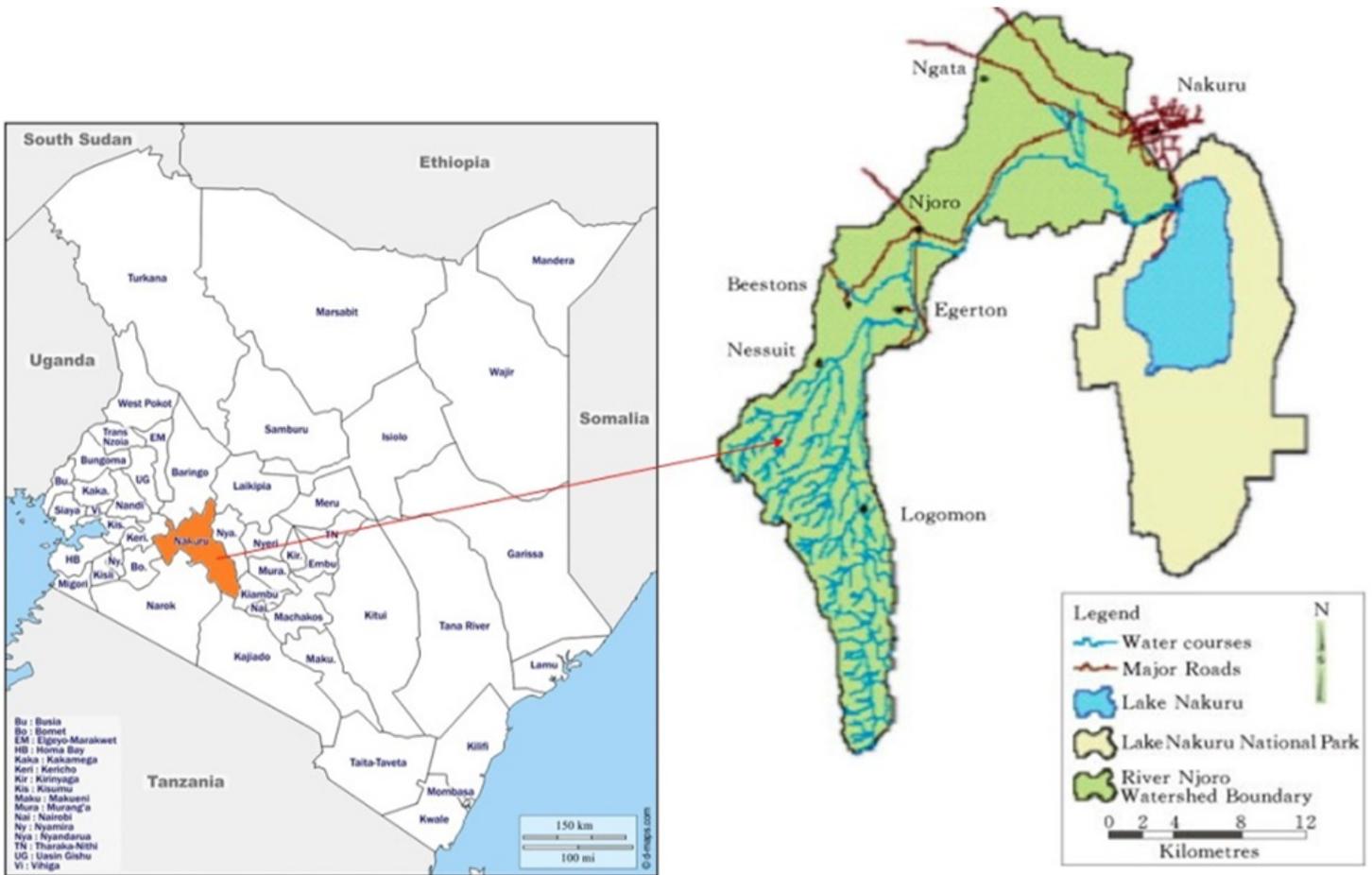


Figure 1

Geographical location of River Njoro water shed in Njoro Sub County. Njoro Sub County is in Nakuru County, Kenya (Map of Kenya shown on the left). River Njoro watershed is situated along River Njoro, which originates from the Eastern part of Mau forest (around Logomon and Nessuit areas) and terminates into Lake Nakuru (Mainuri and Owino 2013) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

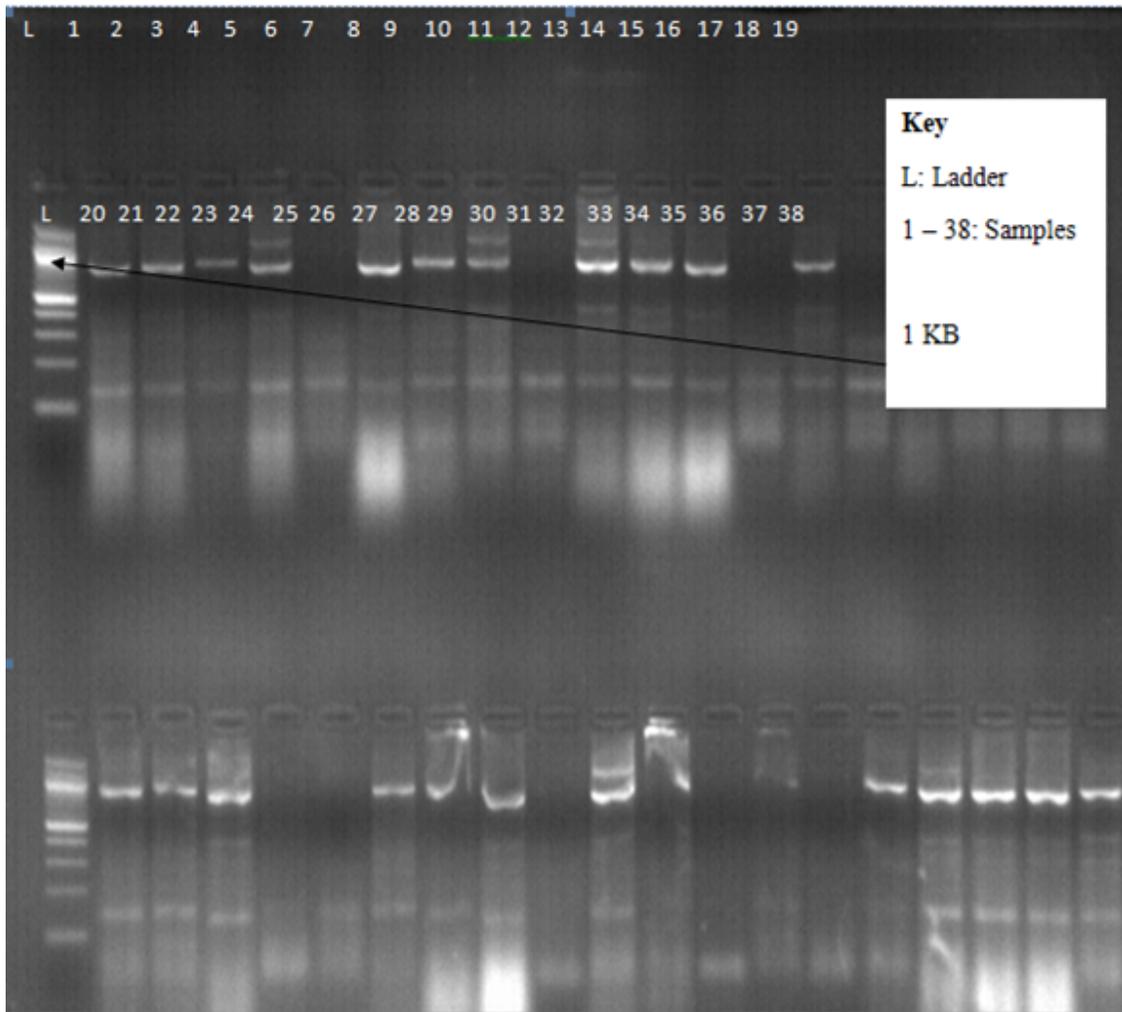


Figure 2

An ethidium bromide stained agarose gel showing DNA fragments produced by PCR amplification of the 18s ribosomal RNA genes extracted from *Cryptosporidium* spp. The amount of genomic DNA used as template was 50ng. Lane 1 contains a 1KB ladder; the DNA fragments of the 38 *Cryptosporidium* samples are labeled 1-38. For the negative control, sterile distilled water was used in place of template DNA

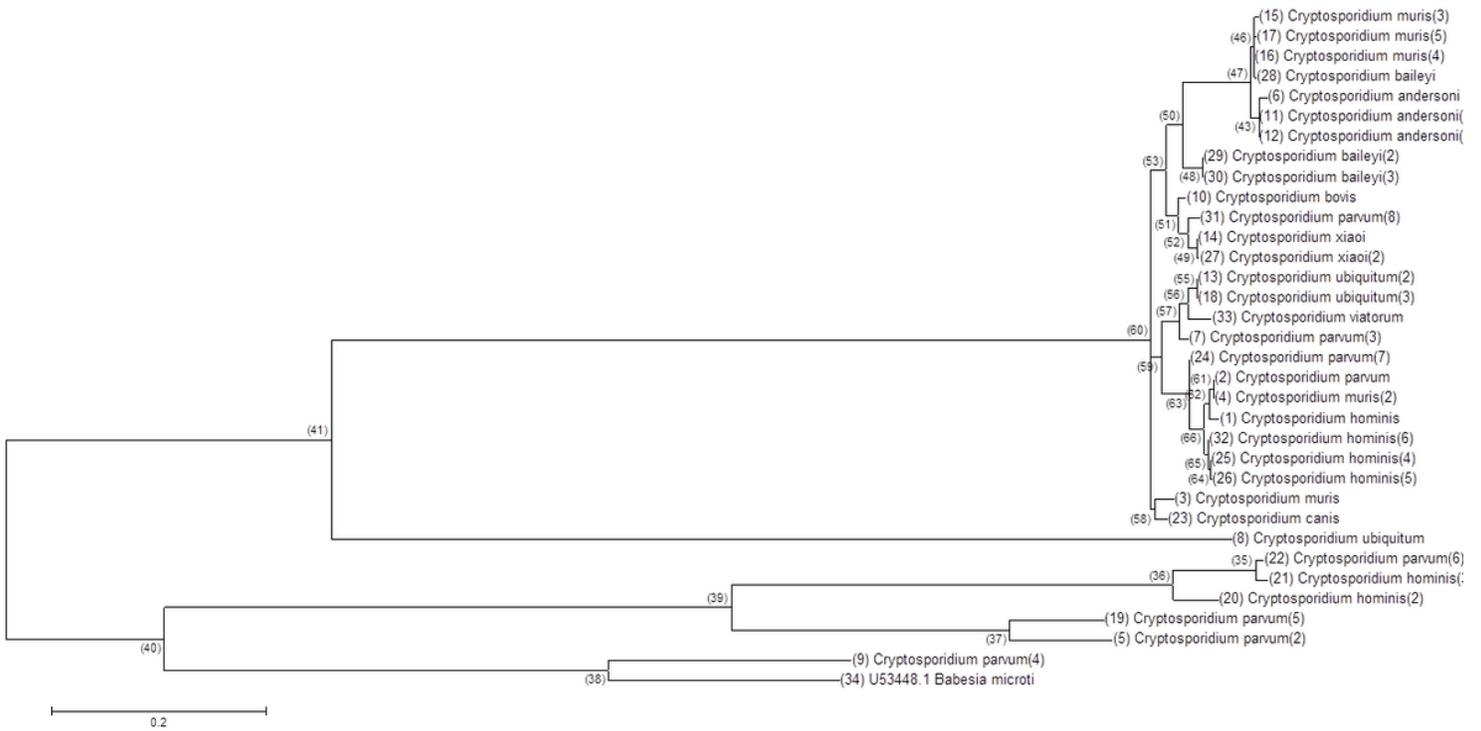


Figure 3

Phylogenetic dendrogram from Maximum Likelihood Analysis of the 33 *Cryptosporidium* sequences identified in Njoro Sub County. *Babesia microti*, an apicomplexa, was identified as an outgroup. The organism analyzed, *Cryptosporidium*, is appropriately grouped into two evolutionary clusters and three sub clusters. The numbers in brackets indicate the bootstrap values