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Review Article

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Cryptosporidium infections in Nepal: A narrative review

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ABSTRACT

Cryptosporidium, a ubiquitous intracellular protozoan parasite, is capable of causing zoonotic infections in humans and domestic and wild animals. The primary aim of this review is to summarize the infection prevalence of *Cryptosporidium* spp. in Nepal. Based on all the available papers published until November 2022, we have also summarized the pattern of diagnostic methods, epidemiological factors, prevalence, and its genotypes distributed in humans, animals and environmental samples (mainly water). Among humans, the overall prevalence of cryptosporidiosis was 7.6% (1 328/17 527), of which the highest prevalence was identified in children (9.0%; 1 225/13 545), followed by HIV patients (4.6%; 51/1 107) and adults (1.8%; 52/2 875). The domestic animals were more infected (23.2%; 252/1 088) than the wild animals (18.2%; 63/347) by *Cryptosporidium*, and water (32.6%; 29/89) remained the major source of environmental contamination by *Cryptosporidium* oocysts. Molecular epidemiological studies regarding the zoonotic transmission of cryptosporidiosis in Nepal are very few. However, molecular techniques have confirmed *Cryptosporidium (C.) parvum* isolates from humans as genotype Ia2, *C. ryanae* in zebu cattle and water buffalo, and *C. ubiquitum* in swamp deer by PCR-RFLP and sequence analysis of SSU rRNA gene. Extensive studies of *Cryptosporidium* at molecular level are warranted to find out its genomic variants in human and other vertebrates, both domestic and wild, in Nepal to elucidate its zoonotic potential in the future.

KEYWORDS: *Cryptosporidium;* Humans; Animals; Water contamination

1. Introduction

Cryptosporidiosis, a food- and water-borne disease, is one of the predominant causes of enteric infection in humans and other vertebrates. *Cryptosporidium* was firstly described by Tyzzer[1] as an intestinal protozoan parasite from the gastric mucosa of mice (*Mus musculus*), and classified as *Cryptosporidium(C.) muris*. It has high risk of infection through faecal-oral route through both anthroponotic and zoonotic transmission cycles owing to its low infectious dose (<10 oocysts) and resistance to various disinfectants including chlorine[2]. Studies have identified several risk factors like poor hygiene, exposure to animals, overcrowding, and unsafe drinking water in relation to *Cryptosporidium* infection in low- and middle-income countries[3,4].

Globally, *Cryptosporidium* is the most common pathogen causing diarrhoea after rota virus in young children[5,6]. *Cryptosporidium* infection leads to a consequence of diarrhea and nutritional deficiencies, making it a significant health problem in immunocompromised persons with HIV infection[4,7,8], but it may result in an asymptomatic course in persons with normal immune systems[9]. Extensive genetic variation within the genus *Cryptosporidium* has been reported. Of the 44 valid *Cryptosporidium*

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species and more than 120 genotypes, until now 19 species and four genotypes have been reported in humans in whom *C. hominis*, *C. parvum*, *C. meleagridis*, *C. canis* and *C. felis* are the most prevalent[10].

Bovine cryptosporidiosis is considered as one of the major causes of diarrhoea and diarrhoea-related deaths in neonatal calves[11]. Four specie of *Cryptosoporidium* such as *C. parvum*, *C. bovis*, *C. andersoni* and *C. ryanae* have been reported to be common species infecting cattle resulting in bovine cryptosporidiosis[12,13]. Cryptosporidiosis cases have also been detected in various species of captive wild animals and wild birds, indicating its wide host range and zoonotic potential[14–16].

Cryptosporidium has high potential for zoonotic dissemination in low- and middle-income countries[4], however, comprehensive epidemiological studies are required to track its transmission dynamics and host specificity[10]. Compared with other countries, very few data are available on the prevalence of *Cryptosporidium*, its genotypes and subtypes in humans and animals in Nepal. Taylor *et al.*[17], first time in Nepal, have reported *Cryptosporidium* in 5% of the residents and travellers to Nepal by acid-fast staining method. Some pioneering studies on prevalence of cryptosporidiosis in Nepal were conducted by Sherchand *et al.*[18], Shrestha *et al.*[19] and Sherchand *et al.*[20]. The aim of this review is to summarize the findings of cryptosporidiosis from communities, fields and hospitals-based studies. Similarly, emphasis was given to find the diagnostic methods practiced mainly in hospitals, epidemiological factors and burden in various groups of people and animals, and recent trend of *Cryptosporidium* related research in context to Nepal. This review has pointed out the research gaps and opportunities for further studies on cryptosporidiosis.

2. Search strategy and selection criteria

We searched PubMed, Web of Science, ResearchGate and Google Scholar with the search terms "*cryptosporidium*", "cryptosporidiosis", "epidemiology", "diagnosis", "Nepal" and collected all the papers available until November 30, 2022. We included 33 relevant papers with citations in English providing detail information regarding diagnostic tools associated to *Cryptosporidium*, disease prevalence either in humans, domestic farm animals, wild animals and/or environmental samples in context to Nepal.

3. Diagnostic methods

3.1. Morphological and immunological tools

Direct microscopy, wet mount, concentration techniques (filtration, flocculation, Sheather's sucrose floatation, immunomagnetic separation, saturated sodium chloride floatation, centrifugation), detection techniques (immunofluorescence microscopy, enzymatic immunoassay, enzyme linked immunosorbent assay), staining techniques, differential interference contrast microscopy, immunochromatographic dip strip test, and cell culture have been practiced by several researchers throughout the globe. However, modified Ziehl-Neelson staining technique followed by microscopy is most widely used by most of the researchers, and for clinical diagnosis of *Cryptosporidium* infections in Nepal. The specificity of staining techniques and antigen detection has remarkable variation while the PCR for *Cryptosporidium* has been found to be highly sensitive[21–26].

3.2. Molecular approach

Various genotyping tools have been developed for exact identification of various species of *Cryptosporodium*. PCR assay targeting the small subunit (SSU) rRNA gene has been used as a common *Cryptosporidium*-genotyping tool[27,28]. Similarly, qPCR-based genotyping assays[29,30], SSU rRNA-based PCR assays using fluorescence resonance energy transfer probes and melt curve analysis can be used for rapid genotyping of *Cryptosporidium* spp. in humans[31], and MboII in RFLP analysis of PCR products from *Cryptosporidium* spp. in ruminants have been applied[32]. Subtyping based on analysis of DNA sequence of the 60 kDa glycoprotein (gp60) has been common in recent days[11,33]. There were merely two reports regarding the molecular surveillance data of *Cryptosporidium* spp. in context to Nepal[34, 35].

4. Reported infections of *Cryptosporidium*

4.1. Cryptosporidium in humans

Several incidences had been reported for potential transmission of the *Cryptosporidium* oocysts among humans mainly through contaminated water sources (tap, well and river water) and animals in Nepal. The study sites as mentioned in the selected papers have been depicted in Figure 1 and the overall studies conducted in human subjects are summarized in Table 1.

We have included 33 studies for analysis. Of the 20 studies conducted among humans, 13 were associated to hospital-based investigations among children, four in HIV seropositive cases from various hospitals and only three studies were conducted in adult humans visiting hospital. Overall 16 studies were based on hospital in-and out-patients, four community-based studies and only one study had combination of both hospital patients and community people. Based on the available studies, a total of 17 527 stool samples from human subjects were analyzed by using concentration techniques and modified Ziehl-Neelsen stain followed by microscopy. *Cryptosporidium* infection prevalence was highest in children (9.0%; 1 225/13 545) followed by HIV cases (4.6%; 51/1 107) and adults (1.8%; 52/2 875), however the overall

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Table 1. *Cryptosporidium* identification in various groups of humans in Nepal.

Categories	Location	Sample size	Sex ratio	Age	Diagnostic methods	Positive (%)	References
Studies in HIV/ AIDS seropositive cases							
	TUTH	128[ł] 72[χ] 100[φ]	M: 108 F: 92 -	- - -	Sheather's sucrose flotation Modified Ziehl-Neelsen stain	8.6% (11/128) 2.8% (2/72) 1.0% (1/100)[φ]	[36]
	MBRB	50	M: 33 F: 17	0–60 years	Sheather's concentration Modified Ziehl-Neelsen stain	18.0% (9/50)	[37]
	NPHL	745	M: 484 F: 261	Median age: 30	Modified Ziehl-Neelsen stain	13.8% (23/167)[*]	[38]
	Kathmandu	112	-	16–52 years	Modified Ziehl-Neelsen stain	5.4% (6/112)[#]	[39]
HIV seropositive cases subtotal		1 107	M: 625 F: 370			4.6% (51/1 107)	
Studies in human adults							
	Kathmandu	328	All male travellers to Nepal	-	Direct smear Formalin-ether concentration Modified Ziehl-Neelsen stain	4.6% (15/328)	[17]
Hospital/ clinics and community based study	Kathmandu	2 483	-	>16 years	Formalin-ethylacetace concentration Modified Ziehl-Neelsen stain	1.4% (36/2 483)	[40]
	SAT	64	-	-	Formal-ether sedimentation Modified Ziehl-Neelsen stain	1.6% (1/64)	[41]
Human adults subtotal		2 875				1.8% (52/2 875)	
Studies in human children							
Children with acute diarrhea	Kathmandu	124	M: 81 F: 43	6–60 months	Direct microscopy Formalin-ether concentration	4.8% (6/124)	[42]
Neighbouring children without diarrhea (Control)		103	-		Modified Ziehl-Neelsen stain	0%	
	TUTH, BktH, KCH, TMC	354	M: 159 F: 195	-	Modified Ziehl-Neelsen stain DMSO stain	6.8% (24/354)	[43]
	KCH	195[♦] 195[▾]	- -	1 month–15 years	Wet mount Modified Ziehl-Neelsen stain	3.1% (6/195) 5.6% (11/195)	[44]
	KCH, BPH	334	M: 210 F: 124	<15 years	Fluorescent antibody technique	2.4% (8/334)[▪]	[45]
	KCH	460	M: 276 F: 184	<15 years	Saline and iodine wet mount Formal-ether sedimentation Sheather's sucrose floatation Modified Ziehl-Neelsen stain	10.4% (48/460)	[46]
Hospital/ clinics and community based study	Kathmandu	6 517	-	<15 years	Formalin-ethylacetace concentration Modified Ziehl-Neelsen stain	15.1% (981/6 517)	[40]

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Categories	Location	Sample size	Sex ratio	Age	Diagnostic methods	Positive (%)	References
Children with persistent diarrhoea	MTH	253	M:147 F:106			0.4% (2/508)$^{\Delta}$	[47]
Children with acute diarrhoea (disease control)		155	M: 87 F: 68	<5 years	Modified Ziehl-Neelsen stain	0%	
Children form community (normal control)		100	M: 55 F: 45			0%	
	BPKIHS	863	-	<10 years	Modified Ziehl-Neelsen stain	4.2% (36/863)	[48]
	Kathmandu	507	-	3–14 years	Formal-ether concentration Acid-fast stain (Kinyoun's method)	0.8% (4/507)	[49]
Diarrhoeal (cases)	KCH, BH	1 200	-	3–60 months	ELISA	2.2% (26/1 200)	[50]
Non-diarrhoeal (controls)	KCH, BH	1 200	-		Formalin ethylacetate sedimentation concentration Modified Ziehl-Neelsen stain	0.8% (10/1 200)	[50]
	TUTH	250	-	<15 years	Modified Ziehl-Neelsen stain	0%	[51]
	Public school and slum areas, Kathmandu	350	-			1.4% (5/350)	
	Kathmandu	187	M: 99 F: 88	3–12 years	Direct microscopy Formal-ether concentration Sheather's sucrose floatation Modified Ziehl-Neelsen stain	29.4% (55/187) 24.2% (24/99) 35.2% (31/88)	[52]
	SAT	198	-	-	Formal-ether sedimentation Modified Ziehl-Neelsen stain	1.5% (3/198)	[41]
Children sub-total		13 545				9.0% (1 225/13 545)	
Grand total		17 527				7.6% (1 328/17 527)	

HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; ART: Antiretroviral Treatment; TUTH: Tribhuvan University Teaching Hospital, Kathmandu; MBRB: Manohara and Bagmati river Basin, Kathmandu; NPHL: Public Health Laboratory of Institute of Medicine; BktH: Bhaktapur Hospital, Bhaktapur; KCH: Kanti Children's Hospital, Kathmandu; TMC: Tripureshwor Medical Clinic, Kathmandu; BPH: Birendra Police Hospital, Bhaktapur; MTH: Manipal Teaching Hospital, Pokhara; BPKIHS: BP Koirala Institute of Health Science, Dharan; BH: Bharatpur Hospital, Chitwan; SAT: Slum Areas of Thapathali, Kathmandu; PEM: Protein energy malnutrition

•Study in premonsoon period

•Study in monsoon period

†HIV cases not taking ART medication

$^{\chi}$HIV cases taking ART medication

$^{\Delta}$1 case from HIV and 1 from PEM sufferer (both had persistent diarrhoea)

•Highest prevalence in summer season (4 cases)

**Cryptosporidium* was detected only in HIV positive cases having <200/μL CD4 T-cell count

$^{\#}$*Cryptosporidium* was detected only in HIV seropositive cases, however study was conducted in both HIV positive and HIV high risk group people

$^{\varphi}$It was HIV seronegative control, and excluded in analysis

prevalence was 7.6% (1 328/17 527).

Cryptosporidiosis is considered to be a major threat to AIDS patients who are deprived of highly active antiretroviral therapy especially in developing countries[53]. In a hospital-based study in western Nepal, *Cryptosporidium* was found to be associated with persistent diarrhoea in HIV infected immunocompromised children, and malnutrition[47]. In Nepal, the prevalence of opportunistic cryptosporidiosis has been reported in HIV seropositive patients[39,54,55] as well as in HIV positive cases seeking for CD4 T-cell count[38,56]. The discrepancy of cryptosporidiosis in rural and urban settings could not be categorized because most of the studies as mentioned above were hospital-based (in-patients and out-patients), and demographic characteristics like rural and urban data were lacking. In the studies conducted in hospital settings in Kathmandu, it can be assumed that most of the samples examined belong to the people from rural setting as many people from rural

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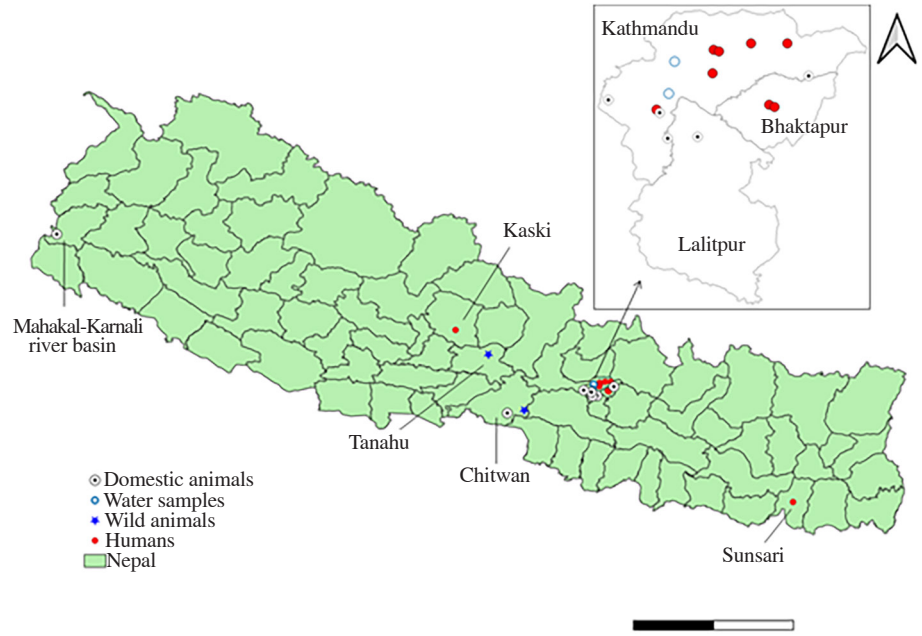

Figure 1. Study sites of *Cryptosporidium* infections in humans, domestic animals, wild animals and water samples in Nepal.

areas visit hospitals in Kathmandu on their own or on a referral basis. Most of the cases have been reported from urban areas as hospitals in urban settings are provided with laboratory facilities for epidemiological studies. However, studies have not segregated the results based on rural and urban areas. A wider surveillance on cryptosporidiosis in various human communities of different ethnic origin is worthwhile in order to estimate the disease burden.

4.2. Cryptosporidium in domestic animals

Of the total 1 088 faecal samples examined from various domestic animals, 23.2% (252/1 088) were found shedding *Cryptosporidium* oocysts. The highest prevalence was recorded in buffalo (47.3%; 98/207), followed by cattle (42.3%; 116/274), cats (11.2%; 12/107), pigs (10%; 10/100) and goats (4.0%; 16/400) (Table 2). Domestic ungulates contribute as potential sources for environmental contamination of *Cryptosporidium* oocysts[57]. Paudyal *et al.* reported overall 32.0% (16/50) calves positive for *Cryptosporidium* oocysts in the periphery of Manohara and Bagmati river basin of Kathmandu[37]. A cross sectional study was conducted among cattle and buffalo calves near the river basins of Mahakali and Karnali, Nepal[58]. Of the total 350 faecal samples tested, 48.6% were positive for *Cryptosporidium* with higher prevalence in cattle calves (50.3%; 72/143) than the buffalo calves (47.3%; 98/207). For both the groups the highest prevalence of infection was recorded in the age group 3-6 months (53.7%; 101/188) and males had a higher prevalence (53.1%; 78/147) than females (45.3%; 92/203). Similarly, in the same study, the exotic breeds had a higher prevalence of infection (50.6%; 82/162) compared to indigenous breeds (46.8%; 88/188), the highest prevalence was observed in Bardiya (57.7%; 75/130) followed by Kanchanpur (46.4%; 64/138) and Kailalil (37.8%; 31/82), and the Karnali river basin with higher incidence (50.0%; 106/212) than the Mahakali river basin (46.4%; 64/138)[58].

Based on questionnaire survey, thatched sheds, more grazing hours and use of surface water were considered to be the probable risk factors of its prevalence[58]. Goats have been incriminated as a probable source for dissemination of cryptosporidial oocysts to the urban areas[59]. As the livestock survey in Nepal comprises a large number of cattle (2 280 542 holdings; 6 430 397 heads), buffaloes (1 668 820 holdings; 3 174 389 heads), yaks/chauri (6 235 holdings; 48 865 heads), sheep (98 464 holdings; 612 884 heads), goats (2 463 253 holdings; 11 225 130 heads) and pigs (477 984 holdings; 870 197 heads)[62], a nationwide surveillance on *Cryptosporidium* sp. is necessary in order to determine the prevalence, genotypes and their zoonotic potential.

4.3. Cryptosporidium in wild animals

Relatively very few studies are carried out on *Cryptosporidium* infection in wild animals in Nepal. A high prevalence was reported in swamp deer (62.5%; 20/32) followed by wild buffaloes (37.0%; 30/81), and the overall prevalence was found to be 18.15% (63/347) based on the studies available (Table 3). Further investigations are essential to unveil the disease burden and the risk of zoonotic transmission.

4.4. Potential water–borne cryptosporidiosis

Water-borne gastrointestinal pathogens are found in high concentrations in the faeces of infected persons as primary source of infection and they are transmitted primarily through the faecal–oral route, mainly through consumption of contaminated food or

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Table 2. *Cryptosporidium* identification in domestic animals in Nepal.

Host animals	Location	Sample number	Sex ratio	Age (Months)	Diagnostic methods	Positive (%)	References
Zebu cattle	Buffer zone of CNP	81	-	2-6	Water-ethyl sedimentation Modified Ziehl-Neelsen stain	34.6% (28/81)	[35]
Cattle calves	Manohara and Bagmati river basin of Kathmandu	50	-	-	Sheather's sucrose concentration	32.0% (16/50)	[37]
					Modified Ziehl-Neelsen stain		
Cattle	River basin of Mahakali and Karnali (Bardiya, Kanchanpur and Kailali)	143	M: 63 F: 80	0-12	Modified Ziehl-Neelsen Stain	50.3% (72/143)	[58]
					Sucrose Floatation for oocysts	58.7% (37/63) 43.8% (35/80)	
Cattle subtotal		274				42.3% (116/274)	
Buffalo		207	M: 84 F: 123		Modified Ziehl-Neelsen Stain Sucrose Floatation for oocysts	47.3% (98/207) 48.8% (41/84) 46.3% (57/123)	
Goat	Kathmandu valley	400	-	-	Wet mount (Saline and iodine) Centrifugation Saturated NaCl floatation Modified Ziehl-Neelsen stain	4.0% (16/400)	[59]
Pigs	Chitwan	100	M: 46 F: 54		Modified Ziehl-Neelsen stain	10.0% (10/100)	[60]
Sucklings and weaners		30				16.7% (5/30)	
Growers		30				10.0% (3/30)	
Adults		40				5.0% (2/40)	
Cats	Chitwan	107			Modified Ziehl-Neelsen stain	11.2% (12/107)	[61]
Grand total		1 088				23.2% (252/1 088)	

water. Food- and water-borne diseases are considered to be the main reason for mortality and morbidity, and they are exacerbated by climate change effects globally[66]. Of the total deaths due to diarrhoea in low- and middle-income countries (60%), inadequate drinking water accounts for 35% followed by sanitation (31%) and hygiene (12%)[67]. About 83% of population in Nepal live in rural setting where more than 90% are devoid of toilet facilities[68], which indicates higher chances of fecal contamination to the water sources. Contamination of manure by *Cryptosporidium* oocysts is a potential source of further contamination of environment and water, and then to humans and animals[69, 70].

The *Cryptosporidium* oocysts positivity ranges from 20% to 50% (highest in river and sewage water) (Table 4). Protozoa and helminth parasites are one of the major causes of diarrhoea in people of all age groups in the Kathmandu Valley, especially during the rainy season (June–September)[71,72]. People residing in the Kathmandu valley especially rely on groundwater from shallow wells for their daily water requirement, but most of these wells do not meet the WHO microbiological guideline values for drinking water[73]. Tanker water seems to be one of the environmental determinants in transmission of cryptosporidiosis and other intestinal parasites in slum areas of Kathmandu[41] as sources of tanker water might be polluted[49]. The risk of acquiring water-borne diarrhoea from *Cryptosporidium* was reported to be higher than the acceptable limit in water resources for drinking and bathing[74]. Knowledge regarding the prevalence of water-borne pathogens is crucial to evaluate the risk of infection from pathogens transmitted via contaminated water. The prevalence study was mainly carried in ground water (wells) and river water in Kathmandu valley[74–76], however epidemic outbreak of food and water borne cryptosporidiosis at a large scale was not reported.

4.5. Molecular characterization of Cryptosporidium infections

C. parvum is mainly composed of two genotypes, I (human genotype or anthroponotic genotype) and II (bovine genotype or zoonotic genotype). The six isolates of *C. parvum* from humans in Nepal were identified as genotypes Ia2 by means of DNA sequencing, PCR-restriction fragment length polymorphism (PCR-RFLP), and RFLP–single-strand conformational polymorphism (RFLP-SSCP) analyses of Cpgp40/15 encoding gene[34]. Feng *et al.*[35] carried out PCR-RFLP and sequence analysis of SSU rRNA gene in order to genotype *Cryptosporidium* specimens from calves

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Table 3. *Cryptosporidium* identification in wild animals in Nepal.

Hosts	Location	Sample number	Age (months)	Diagnostic methods	Positive (%)	References
Swamp deer	Buffer zone of CNP	32	-	Water-ethyl sedimentation Modified Ziehl-Neelsen stain	62.5% (20/32)	[35]
Wild buffalo	Buffer zone of CNP	81	2-7	Modified Ziehl-Neelsen stain	37.0% (30/81)	
Himalayan goral	Tanahu	19	-	Modified Ziehl-Neelsen stain	26.3% (5/19)	[63]
Pigeons[#]	Chitwan	155		Modified Ziehl-Neelsen stain	3.2% (5/155)	
	Residential area	65			6.2%(4/65)	[64]
	Temple area	90			1.1% (1/90)	
Bats		-				[65]
Insectivorous bats	Chitwan	30		Modified Ziehl-Neelsen stain	10% (3/30)	
Frugivorous bats		30			0%	
Total		347			18.2% (63/347)	

CNP: Chitwan National Park

[#]Pigeons have been considered under wildlife category as they are not commercially farmed in the study site.

Table 4. *Cryptosporidium* identification in water samples in Nepal.

Locations	Sample number	Diagnostic methods	Positive (%)	References
Residential areas in Kathmandu and around Bagmati river	9 (ground water from shallow wells)	Immunomagnetic separation (IMS) Fluorescent staining Fluorescence microscopy	11.1% (1/9) [with 2.2 oocysts/L]	[75]
Kathmandu valley	1 (River water)	Immunomagnetic separation (IMS)	100.0% (1/1) [140 oocysts/L]	[74]
	22 (Dug wells)		31.8 % (7/22)	
	15 (Tube wells)	Immunofluorescence assay	7.0% (1/15)	
Bagmati river	18 (Surface water)	Fluorescence microscopy	38.9% (7/18)	[76]
Kathmandu	24	Fluorescence microscopy	50.0% (12/24)[*]	[77]
Total	89		32.6% (29/89)	

[*]Only positive results for *Cryptosporidium* of river and sewage water sample were shown.

of zebu cattle and water buffalo, and swamp deer (*Cervus duvauceli*) from the buffer zone of Chitwan National Park, Nepal (Table 5). The *C. ryanae* variant reported in this study from zebu cattle indicates a unique cryptosporidiosis transmission in bovine animals and also deserves the possibility of cross-species transmission between allied host species sharing the same habitats.

4.6. Research gap, challenges and opportunities of Cryptosporidium research in Nepal

Research has confirmed that *Cryptosporidium* is one of the leading causes of food- and water-borne diarrhoea in children[5,30], adults, immunocompetent and immunocompromised individuals[7], and domestic and wild animals[14,35]. However, reports related to geriatrics, kidney patients, diabetics and organ transplant recipient with continuous use of immunosuppressants are lacking. In Nepal, most of the reports are related to hospital-based studies mainly for clinical diagnosis of diarrheal cases and very negligible number in community level. As more than 80% of Nepalese are relying on agriculture, the potential of zoonotic transmission of such parasites through animal excreta, soil, food and water are very common. Similarly, extensive research with epidemiological design are lacking in humans and wild life.

Despite the interest of researchers to use recent molecular diagnostic tools for the diagnosis and genomic identification of *Cryptosporidium* strains, most of the studies are relying on concentration method, wet mount and mostly modified Ziehl-Neelsen staining. The sensitivity and specificity of various diagnostic tools are different. The staining techniques (Ziehl-Neelsen and Safranine methylene blue) are less sensitive for detection of *Cryptosporidium* in HIV seropositive patients with CD4 count >200 cells/L. This finding suggests the chances of missing of cryptosporidiosis in patients who have CD4 count >200 cells/L, if only Ziehl-Neelsen and Safranine methylene staining technique is employed[21]. But these techniques are common practice in most of the teaching hospitals and universities in Nepal. The use of established molecular technique for species identification was very rare. The lack of awareness, education and fundamental health hygiene are predisposing factors for the seasonal outbreak of food- and water-borne diseases mainly in the rural areas. Most of the reports on cryptosporidiosis related diarrhoea are diagnosed only up to generic level. Therefore, diagnosis up to the species level and molecular characterization in relation to zoonotic potential are scarce. The chance of cross-species transmission between different species of domestic animals, wild animals to domestic animals or to the humans specially to the farmers or shepherds through pasture and water resource contamination cannot be ruled out. The existing drainage and pipe line system for distribution of drinking water in the urban regions are posing threat of fecal contamination. In addition to this, the trend of direct disposal of human excreta without treatment to the nearby streams and rivers also favour several

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Table 5. Genotypes of *Cryptosporidium* isolates from humans and other vertebrates in Nepal.

Hosts	Age (month)	Sample number	Positive by microscopy	Molecular typing	Genotype	References
Humans	-	6 isolates of *C. parvum*	-	Intraspecies polymorphism analysis based on RFLP-SSCP analysis of Cpgp40/15 gene	Genotype Ia2*	[34]
Zebu calves	2-6	81	12	RFLP and sequencing	*C. rynae*	[35]
Water buffalo calves	2-7	81	16		*C. rynae*	
Swamp deer	-	32	4		*C. ubiquitum*	

*All isolates were found to be Genotype Ia2, however, previously it was recognized as *Cryptosporidium parvum* genotype I, and now it is usually recognized as *C. hominis*.

water-borne diseases including cryptosporidiosis[78]. Presence of *Cryptosporidium* oocysts in water samples increases the chances of zoonotic transmission[79]. The presence of livestocks at home, use of untreated drinking water, habit of consuming raw fruits and vegetables, and poor sanitation are incriminated to the occurrence of *Cryptosporidium* in the low-income communities of Kathmandu[51]. Even if the drinking water is treated with chlorination at distribution centre or at home it is not certain to completely prevent the chance of infection of *Cryptosporidium* oocysts owing to its chlorine resistance. It is an utmost importance from the concerned authorities and research institutions to address the public health issues considering recent trend of urbanization, movement of the public, transportation of animal breeds within the country or cross border transport. At the same time, the use of integrated advanced molecular diagnostic tools along with well prepared and designed epidemiological field studies of cryptosporidiosis with participation of research scholars and scientists within the country as well as collaborative approach with foreign researchers are necessary so as to dig out the real scenario of its molecular diversity.

5. Conclusions

The domestic animals are more infected (23.2%) than the wild animals (18.2%) followed by humans (7.6%), and water has been noticed as one of the major sources of environmental contamination by *Cryptosporidium* oocysts in urban areas. Most of the studies are based on microscopic observation in clinical specimens. Molecular epidemiological evidences regarding the zoonotic transmission of cryptosporidiosis in Nepal are negligible. Some studies carried out in domestic animals and wild animals in Nepal indicated that there could be more genotypes of *Cryptosporidium* with zoonotic potential. The role of domestic animals, wild animals and environmental determinants in transmission of cryptosporidiosis needs to be investigated in context to Nepal. Therefore, an integrated collaboration among parasitologists, molecular biologists, clinicians, veterinarians and epidemiologists is necessary to better understand the genetic variants of *Cryptosporidium* in humans, domestic animals and wild animals, and their zoonotic potential in Nepal.

Conflict of interest statement

The authors claim there is no conflict of interest.

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Authors' contributions

All the authors discussed and designed the study. Dhakal P collected, screened and analysed the literatures. Dhakal P drafted the first manuscript. Li J and Zhang L edited and finalized the manuscript. All the authors have read and approved the final version to publish.

References

[1] Tyzzer EE. A sporozoan found in the peptic glands of the common mouse. *Proc Soc Exp Biol Med* 1907; **5**: 12-13.

[2] Helmy YA, Hafez HM. Cryptosporidiosis: From prevention to treatment, a narrative review. *Microorganisms* 2022; **10**(12): 2456.

[3] Bouzid M, Kintz E, Hunter PR. Risk factors for *Cryptosporidium* infection in low and middle income countries: A systematic review and metaanalysis. *PLoS Negl Trop Dis* 2018; **12**: e0006553.

[4] Yang X, Guo Y, Xiao L, Feng Y. Molecular epidemiology of human cryptosporidiosis in low- and middle-income countries. *Clin Microbiol Rev* 2021; **34**(2): e00087-19.

[5] Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, et al. Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: A meta-analyses study. *Lancet Glob Health* 2018; **6**(7): e758-e768.

[6] Levine MM, Nasrin D, Acácio S, Bassat Q, Powell H, Tennant SM, et al. Diarrhoeal disease and subsequent risk of death in infants and children residing in low-income and middle-income countries: Analysis of the

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]

GEMS case-control study and 12-month GEMS-1A follow-on study. *Lancet Glob Health* 2020; **8**(2): e204-e214.

[7] Wang RJ, Li JQ, Chen YC, Zhang LX, Xiao LH. Widespread occurrence of *Cryptosporidium* infections in patients with HIV/AIDS: Epidemiology, clinical feature, diagnosis, and therapy. *Acta Trop* 2018; **187**: 257-263.

[8] Omolabi KF, Odeniran PO, Soliman ME. A meta-analysis of *Cryptosporidium* species in humans from southern Africa (2000-2020). *J Parasit Dis* 2022; **46**(1): 304-316.

[9] Savaş Şen Z, Ta ar MA, Demirel Kaya F, Güne A, Vezir E, Hızarcıo lu Gül en H, et al. A rare complication of acute diarrhae caused by *Cryptosporidium*: Possible hepatobiliary system involvement in a child without immunodeficiency. *Mikrobiyol Bul* 2019; **53**(4): 464-471.

[10]Ryan U, Zahedi A, Feng Y, Xiao L. An update on zoonotic *Cryptosporidium* species and genotypes in humans. *Animals (Basel)* 2021; **11**(11): 3307.

[11]Wu Y, Zhang K, Zhang Y, Jing B, Chen Y, Xu C, et al. Genetic diversity of *Cryptosporidium parvum* in neonatal dairy calves in Xinjiang, China. *Pathogens* 2020; **9**(9): 692.

[12]Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol* 2018; **34**(11): 997-1011.

[13]Yang X, Huang N, Jiang W, Wang X, Li N, Guo Y, et al. Subtyping *Cryptosporidium* ryanae: A common pathogen in bovine animals. *Microorganisms* 2020; **8**(8): 1107

[14]Zhang K, Zheng S, Wang Y, Wang K, Wang Y, Gazizova A, et al. Occurrence and molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Blastocystis* sp. in captive wild animals in zoos in Henan, China. *BMC Vet Res* 2021; **17**(1): 332.

[15]Liao C, Wang T, Koehler AV, Hu M, Gasser RB. *Cryptosporidium* of birds in pet markets in Wuhan city, Hubei, China. *Curr Res Parasitol Vector Borne Dis* 2021; **1**: 100025.

[16]Lu Y, Pu T, Ma B, Wang L, Zhou M, Chen Y. et al. A survey of *Cryptosporidium* prevalence among birds in two zoos in China. *Peer J* 2022; **10**: e12825.

[17]Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL, Echeverria, P. Etiology of diarrhea among travelers and foreign residents in Nepal. *JAMA* 1988; **260**(9): 1245-1248.

[18]Sherchand JB, Larsson S, Shrestha MP, Shrestha S, Sharma PR, Adhikari R. An incidence of Rotavirus and enteric adenovirus diarrhea, Kanti Children Hospital and general practitioners in the Kathmandu area. *JNMA J Nepal Med Assoc* 1992; **30**: 149- 159.

[19]Shrestha S, Larsson S, Serchand J, Shrestha S. Bacterial and cryptosporidial infection as the cause of chronic diarrhoea in patients with liver disease in Nepal. *Trop Gastroenterol* 1993; **14**(2): 55-58.

[20]Sherchand JB, Shrestha MP, Larsson S, Irai KH, Abe A, Nakanishi M, et al. *Cryptosporidium* infection and diarrhoea in children from Jomsome, Chitwan and Kathmandu valley of Nepal. *Zoonosis Bulletin* 1995; **2**: 17-22.

[21]Kaushik K, Khurana S, Wanchu A, Malla N. Evaluation of staining techniques, antigen detection and nested PCR for the diagnosis of cryptosporidiosis in HIV seropositive and seronegative patients. *Acta Trop* 2008; **107**(1): 17.

[22]Bhat SA, Dixit M, Juyal PD, Singh NK. Comparison of nested PCR and microscopy for the detection of cryptosporidiosis in bovine calves. *J Parasit Dis* 2014; **38**(1): 101–105.

[23]Uppal B, Singh O, Chadha S, Jha AK. A comparison of nested PCR assay with conventional techniques for diagnosis of intestinal cryptosporidiosis in AIDS cases from northern India. *J Parasitol Res* 2014; **2014**: 706105.

[24]Ezzaty Mirhashemi M, Zintl A, Grant T, Lucy FE, Mulcahy G, De Waal T. Comparison of diagnostic techniques for the detection of *Cryptosporidium* oocysts in animal samples. *Exp Parasitol* 2015; **151-152**: 14-20.

[25]Jaiswal V, Brar APS, Sandhu BS, Singla LD, Narang D, Leishangthem GD, et al. Comparative evaluation of various diagnostic techniques for detection of *Cryptosporidium* infection from the faecal samples of diarrhoeic bovine calves. *Iran J Vet Res* 2022; **23**(3): 247-254.

[26]Atwal HK, Zabek E, Bidulka J, DuCharme A, Pawlik M, Himsworth CG. A comparison of assays for the detection of Cryptosporidium parvum in the feces of scouring calves. *J Vet Diagn Invest* 2022; **34**(2): 284-287.

[27]Guy RA, Yanta CA, Bauman CA. Molecular identification of *Cryptosporidium* species in Canadian post-weaned calves and adult dairy cattle. *Vet Parasitol Reg Stud Rep* 2022; **34**: 100777.

[28]Murnik LC, Daugschies A, Delling C. *Cryptosporidium* infection in young dogs from Germany. *Parasitol Res* 2022; **121**(10): 2985-2993.

[29]Boughattas S, Behnke JM, Al-Sadeq D, Ismail A, Abu-Madi M. *Cryptosporidium* spp., prevalence, molecular characterisation and socio-demographic risk factors among immigrants in Qatar. *PLoS Negl Trop Dis* 2019; **13**(10): e0007750.

[30]Johansen ØH, Abdissa A, Bjørang O, Zangenberg M, Sharew B, Alemu Y, et al. Oocyst shedding dynamics in children with cryptosporidiosis: A prospective clinical case series in Ethiopia. *Microbiol Spectr* 2022; **10**(4): e0274121.

[31]Li N, Neumann NF, Ruecker N, Alderisio KA, Sturbaum GD, Villegas EN, et al. Development and evaluation of three real-time PCR assays for genotyping and source tracking *Cryptosporidium* spp. in water. *Appl Environ Microbiol* 2015; **81**(17): 5845–5854.

[32]Feng Y, Ortega Y, He G, Das P, Xu M, Zhang X, et al. Wide geographic distribution of *Cryptosporidium* bovis and the deer-like genotype in bovines. *Vet Parasitol* 2007; **144**(1-2): 19.

[33]Feng Y, Xiao L. Molecular epidemiology of cryptosporidiosis in China. *Front Microbiol* 2017; **8**: 1701.

[34]Wu Z, Nagano I, Boonmars T, Nakada T, Takahashi Y. Intraspecies polymorphism of *Cryptosporidium parvum* revealed by PCR-restriction fragment length polymorphism (RFLP) and RFLP-single-strand conformational polymorphism analyses. *Appl Environ Microbiol* 2003; **69**(8): 4720-4726.

[35]Feng Y, Karna SR, Dearen TK, Singh DK, Adhikari LN, Shrestha A, et al. Common occurrence of a unique *Cryptosporidium ryanae* variant in zebu cattle and water buffaloes in the buffer zone of the Chitwan National

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]

Park, Nepal. *Vet Parasitol* 2012; **185**(2-4): 309-314.

[36]Basnet A, Sherchan B, Rijal BP, Sharma S, Khadga PK. Detection of coccidian parasite and their clinical manifestation, treatment and prophylaxis in HIV infected patients in Tribhuvan University Teaching Hospital. *Scientific World* 2010; **8**: 51-55.

[37]Paudyal S, Shrestha SP, Mahato N. Zoonotic aspects of cryptosporidiosis in Nepal. *Int J Appl Sci Biotechnol* 2013; **1**(2): 21-26.

[38]Tiwari BR, Ghimire P, Malla S, Sharma B, Karki S. Intestinal parasitic infection among the HIV-infected patients in Nepal. *J Infect Dev Ctries* 2013; **7**(7): 550-555.

[39]Adhikari NA, Rai SK, Singh A, Dahal S, Ghimire G. Intestinal parasitic infections among HIV seropositive and high risk group subjects for HIV infection in Nepal. *Nepal Med Coll J* 2006; **8**(3): 166-170.

[40]Ghimire TR, Mishra PN, Sherchand JB. The seasonal outbreaks of *Cyclospora* and *Cryptosporidium* in Kathmandu, Nepal. *J Nepal Health Res Counc* 2005; **3**: 39-48.

[41]Bhattachan B, Sherchand JB, TandukarS, Dhoubhadel BG, Gauchan L, Rai, G. Detection of *Cryptosporidium parvum* and *Cyclospora* cayetanensis infections among people living in a slum area in Kathmandu valley, Nepal. *BMC Res Notes* 2017; **10**(1): 464.

[42]Hoge CW, Echeverria P, Rajah R, Jacobs J, Malthouse S, Chapman E, et al. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. *J Clin Microbiol* 1995; **33**(11): 3058-3060.

[43]Sherchand J, Shrestha M. Prevalence of *Cryptosporidium* infection and diarrhoea in Nepal. *J Diarrhoeal Dis Res* 1996; **14**(2): 81-84.

[44]Ode Y, Sherchand JB, Gurung CK. *Cryptosporidium* and *Cyclospora* studies in two seasons at Kanti children hospital kathmandu Nepal. *Parasitol Int* 1998; (47): 166.

[45]Ono K, Rai SK, Chikahira M, Fujimoto T, Shibata H, Wada Y, et al. Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal. *Southeast Asian J Trop Med Public Health* 2001; **32**(3): 520-526.

[46]Dhakal DN, Rajendra KB, Sherchand J, Mishra P. *Cryptosporidium parvum*: An observational study in Kanti children hospital, Kathmandu, Nepal. *J Nepal Health Res Counc* 2004; **2**(1): 1-5.

[47]Mukhopadhyay C, Wilson G, Pradhan D, Shivananda PG. Intestinal protozoal infestation profile in persistent diarrhea in children below age 5 years in western Nepal. *Southeast Asian J Trop Med Public Health* 2007; **38**(1): 13.

[48]Amatya R, Poudyal N, Gurung R, Khanal B. Prevalence of *Cryptosporidium* species in paediatric patients in Eastern Nepal. *Trop Doct* 2011; **41**(1): 36-37.

[49]Bhandari D, Tandukar S, Sherchand SP, Thapa P, Shah PK. *Cryptosporidium* infection among the school children of Kathmandu Valley. *J Instit Med* 2015; **37**(1): 82-87

[50]Bodhidatta L, Wongstitwilairoong B, Khantapura P, Shrestha SK, Wangchuk S, Pant AR, et al. Cryptosporidiosis: Prevalence in children in Nepal and Bhutan. *J Vet Med Res* 2016; **3**(4):1059; 1-5.

[51]Dhital S, Pant ND, Neupane S, Khatiwada S, Gaire B, Sherchand JB, Shrestha P. Prevalence of enteropathogens in children under 15 years of age with special reference to parasites in Kathmandu, Nepal: A cross sectional study. *Springerplus* 2016; **5**(1): 1813.

[52]Sherchand SP, Joshi DR, Adhikari N, Poudel RC, Pant KP, KC M, et al. Prevalence of cryptosporidiosis among school going children in Kathmandu, Nepal. *EC Microbiol* 2016; **4**: 641-646.

[53]Ahmadpour E, Safarpour H, Xiao L, Zarean M, Hatam-Nahavandi K, Barac A, et al. Cryptosporidiosis in HIV-positive patients and related risk factors: A systematic review and meta-analysis. *Parasite* 2020; **27**:27.

[54]Sharma S, Dhungana GP, Pokharel BM, Rijal BP. Clinical features of HIV/AIDS and various opportunistic infections in relation to antiretroviral status among HIV seropositive individuals from Central Nepal. *Kathmandu Univ Med J (KUMJ)* 2009; **7**(4): 355-359.

[55]Humagain S. Study of opportunistic and other intestinal parasitic infections in relation to CD4 count among human immuno deficiency virus positive patients. *Int J Infect Dis* **2018**: 3-398.

[56]Ghimire A, Bhandari S, Tandukar S, Amatya J, Bhandari D, Sherchand JB. Enteric parasitic infection among HIV-infected patients visiting Tribhuvan University Teaching Hospital, Nepal. *BMC Res Notes* 2016; **9**(1): 1-5.

[57]Hatam-Nahavandi K, Ahmadpour E, Carmena D, Spotin A, Bangoura B, Xiao L. *Cryptosporidium* infections in terrestrial ungulates with fours on livestock: A systematic review and meta-analysis. *Parasit Vectors* 2019; **12**(1): 453.

[58]Bohara TP. *Prevalence of Cryptosporidium in livestock reared near Mahakali and Karnali river basins of Western Nepal. Technical report: Feed the future innovation lab for collaborative research on adapting livestock systems to climate change, TRB–23–2015.* [Online] Available from: https://www.researchgate.net/publication/317343875_ Prevalence_ of_ Cryptosporidium_in_Livestock_reared_near_Mahakali_and_Karnali_ River_Basins_of_Western_Nepal. [Accessed on 19 November 2022].

[59]Ghimire TR, Bhattarai N. A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal. *J Parasit Dis* 2019; **43**(4): 686-695.

[60]Adhikari RB, Dhakal MA, Thapa S, Ghimire, TR. Gastrointestinal parasites of indigenous pigs (Sus domesticus) in south-central Nepal. *Vet Med Sci* 2021; **7**(5): 1820-1830.

[61]Adhikari RB, Dhakal MA, Ale PB, Regmi GR, Ghimire TR. Survey on the prevalence of intestinal parasites in domestic cats (*Felis catus Linnaeus*, 1758) in central Nepal. *Vet Med Sci* 2023; **9**(2): 559-571.

[62]Livestock Statistics of Nepal 2017 (National Report). *Government of Nepal, Ministry of Livestock Development Planning, Monitoring and Evaluation Division, Kathmandu, Nepal. July, 2017.* [Online] Available from:https://nepalindata.com/resource/LIVESTOCK-STATISTICS-OF-NEPAL-2077-78--2020-21 [Accessed on 18 November 2022].

[63]Adhikari J, Adhikari R, Bhattarai B, Thapa T, Ghimire T. Short Communication: A small-scale coprological survey of the endoparasites in the Himalayan goral Naemorhedus goral (Hardwick, 1825) in Nepal.

[Downloaded free from http://www.johb.info on Wednesday, September 27, 2023, IP: 112.66.109.236]

Biodiversitas 2021; **22**: 1285-1290.

[64]Adhikari RB, Ale PB, Dhakal, MA, Ghimire, TR. Prevalence and diversity of intestinal parasites in household and temple pigeons (Columba livia) in central Nepal. *Vet Med Sci* 2022; **8**(4): 1528-1538.

[65]Adhikari RB, Maharjan M, Ghimire TR. Prevalence of gastrointestinal parasites in the frugivorous and the insectivorous bats in Southcentral Nepal. *J Parasitol Res* 2020; **2020**: 8880033.

[66]Cissé G. Food-borne and water-borne diseases under climate change in low- and middle-income countries: Further efforts needed for reducing environmental health exposure risks. *Acta Trop* 2019; **194**: 181-188.

[67]Prüss-Ustün A, Wolf J, Bartram J, Clasen T, Cumming O, Freeman MC, et al. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. *Int J Hyg Environ Health* 2019; **222**(5): 765-777.

[68]National Population and Housing Census 2011 (National Report). *Government of Nepal, National Planning Commission Secretariat, Central Bureau of Statistics, Kathmandu, Nepal. November, 2012.* [Online] Available from: https://unstats.un.org/unsd/demographic/sources/ census/ wphc/Nepal/Nepal-Census-2011-Vol1.pdf. [Accessed on 20 November 2022].

[69]Lasprilla-Mantilla MI, Wagner V, Pena J, Frechette A, Thivierge K, Dufour S, et al. Effects of recycled manure solids bedding on the spread of gastrointestinal parasites in the environment of dairies and milk. *J Dairy Sci* 2019; **102**(12): 11308-11316.

[70]Berhanu K, Ayana D, Megersa B, Ashenafi H, Waktole H. *Cryptosporidium* in human-animal-environment interphase at Adama and Asella areas of Oromia regional state, Ethiopia. *BMC Vet Res* 2022; 18(1), 402.

[71]Uga S, Rai SK, Kimura K, Rai G, Kimura D, Wakasugi M, et al. Parasites detected from diarrheal stool samples collected in Napal. *Southeast Asian J Trop Med Public Health* 2004; **35**: 19-23.

[72]Kimura K, Rai SK, Rai G, Insisiengmay S, Kawabata M, Karanis P, et al. Study on *Cyclospora* cayetanensis associated with diarrheal disease in Nepal and Lao PDR. *Southeast Asian J Trop Med Public Health* 2005; **36**(6): 1371.

[73]Prasai T, Lekhak B, Joshi DR, Baral MP. Microbiological analysis of drinking water of Kathmandu Valley. *Scientific World* 2007; **5**: 112-114.

[74]Shrestha S, Haramoto E, Malla R, Nishida K. Risk of diarrhoea from shallow groundwater contaminated with enteropathogens in the Kathmandu Valley, Nepal. *J Water Health* 2015; **13**(1): 259-269.

[75]Haramoto E, Yamada K, Nishida K. Prevalence of protozoa, viruses, coliphages and indicator bacteria in groundwater and river water in the Kathmandu Valley, Nepal. *Trans R Soc Trop Med Hyg* 2011; **105**(12): 711-716.

[76]Tandukar S, Sherchand JB, Bhandari D, Sherchan SP, Malla B, Ghaju Shrestha R, et al. Presence of human enteric viruses, protozoa, and indicators of pathogens in the Bagmati River, Nepal. *Pathogens* 2018; **7**(2): 38.

[77]Shrestha S, Haramoto E, Sherchand JB, Rajbhandari S, Prajapati M, Shindo J. Seasonal variation of microbial quality of irrigation water in different sources in the Kathmandu Valley, Nepal. *Naresuan Univ Engineer J* 2016; **11**: 57-62

[78]Mishra BK, Regmi RK, Masago Y, Fukushi K, Kumar P, Saraswat C. Assessment of Bagmati river pollution in Kathmandu Valley: Scenario-based modeling and analysis for sustainable urban development. *Sustainabil Water Qual Ecol* 2017; **9**: 67-77.

[79]Kumar T, Abd Majid MA, Onichandran S, Jaturas N, Andiappan H, Salibay CC, et al. Presence of *Cryptosporidium parvum* and *Giardia lamblia* in water samples from Southeast Asia: Towards an integrated water detection system. *Infect Dis Poverty* 2016; **5**: 3.

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