Multiplex PCR for detection of the predominant diarrhea-causing protozoa (*Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum*) in fecal samples of cattle from Babylon Province, Iraq

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Abstract

The work, here, was focused on the detection of the predominant diarrhea-causing protozoa (*Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum*) in fecal samples of cattle from Babylon province, Iraq. Fifty fecal samples from 50 cattle were collected and processed using a specific multiplex polymerase chain reaction (mPCR) technique that employed the small subunit ribosomal RNA (SSrRNA) gene as a molecular target. Using specific primers for each protozoan, the results showed positive identification of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in 38 (76%), 22 (44%), and 12 (24%), respectively, of the fecal samples. The data from the current investigation identify the presence of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in the fecal samples collected from cattle located in Babylon province, Iraq.

Keywords: *Cattle, Cryptosporidium parvum, Entamoeba histolytica, feces, Giardia intestinalis, multiplex PCR, protozoa.*

Introduction

Giardia intestinalis, also known as Giardia duodenalis, is a well-recognized protozoan that causes important infections in human and animals. The microorganism was detected in fecal samples of bovine and dairy cattle from various countries of the world (1–5). Clinical diseases caused by G. intestinalis in cattle have various symptomatic pictures; however, these could be ranged from no signs appeared on the infected animals to the presence of different types of diarrhea including mucoid and fatty feces, persistent diarrhea, and reduction of weight and growth. The infection of giardiasis can be transmitted to humans through cattle-human direct contact and/or contamination of public water supplies (6–10).

In the case of E. histolytica, the protozoan causes amoebiasis which is an important parasitic disease that infects 500 million people with mortalities that can reach up to 40,000 to 100,000 deaths per year. Especially in countries with poor health systems, different infection sources are known for the disease; however, water contaminated with infected human and animal wastes play important roles in spreading the disease [(11–16)].

Cattle are considered as a major source for infections caused by C. parvum inducing a diarrheal disease in humans and loss of production in the infected animals. Also here, contaminated water supplies with feces from infected animals are well-known sources for spreading the infection. [17]

Materials and Method

Sample collection

Fifty fecal samples from 50 cattle were collected from multiple fields of cattle in Babylon province, Iraq. The feces were aseptically collected, placed in zip logs, and transferred in an ice box to a laboratory to perform the molecular analyses.

Genomic DNA extraction

The Stool DNA extraction Kit (Bioneer. Korea) was utilized to extract the genomic DNA (gDNA) from the fecal samples. The obtained DNA was read, using a NanoDrop, for identifying its quality and quantity. Then, the DNA was stored at -20°C in a deep freezer.

Polymerase chain reaction

The samples were processed using a specific mPCR technique that employed SSrRNA gene as a molecular target. Using specific primers, table 1, that were designed via the use of NCBI-Genebank and Primer3plus and purchased from (Bioneer Company, Korea).

Protozoan	Primer sequence (5'-3')		Amplicon size (bp)
G. intestinalis	F	CTGGCCCAAGAGTCCTCAAG	488
	R	CCGGAGTCGAACCCTGATTC	
E. histolytica	F	ATTGGAGGGCAAGTCTGGTG	389
	R	AAATGCTTTCGCTCTCGTGC	
C. parvum	F	ATTGGAGGGCAAGTCTGGTG	242
	R	CCATGCTGGAGTATTCAAGGC	

Then, the AccuPower® PCR PreMix kit (Bioneer, Korea) was employed to prepare the master mix. The tubes of the kit containing a freeze-dried pellet which is the premix components; 1U DNA polymerase, 250µM dNTPs, 10mM Tris-HCl (pH 9.0), 30mM KCl, 1.5mM $MgCl_2$, a stabilizer, and a tracking dye). Using a total volume of 20µl, the master mix was prepared according to the instructions of the kit and contained DNA template at 5µl, each primer direction at 1.5µl (10pmole), and deionized water to complete the volume up to 20µl. The conditions of the reaction inside a thermocycler (Mygene/Bioneer, Korea) were 1 cycle of 5min at 95°C to perform initial denaturation, 30 cycles of (30s at 95°C to generate denaturation, 30s at 58°C to produce annealing, and 1min at 72°C to perform extension), and 5min at 72°C for final extension. Using an ethidium-bromide-pre-treated 2% agarose gel, the PCR products were electrophoresed and screened using a UV-light based imager.

Results

The results showed positive identification of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in 38 (76%), 22 (44%), and 12 (24%), respectively, of the fecal samples, figure 1, 2, and 3, respectively.

Discussion

Giardia intestinalis is a well-recognized protozoan that causes important infections in human and animals. The current results showed the presence of the protozoan in the fecal samples of cattle in the tested regions of Babylon province, Iraq. These findings agree with (1–5) who detected G. intestinalis in fecal samples of bovine and dairy cattle from various countries of the world. Giardiasis is a zoonotic disease that can be transmitted from different infected animals such as cattle to humans via direct contact with infected cattle and/or their feces and using contaminated public water supplies with this microorganism (9,18–23). The current results match up with those observed by 24 who identified the presence of G. intestinalis in fecal samples of cattle from Urmia, northwest of Iran in a rate of 9.34% using different molecular techniques other than the current one. The differences between the current study detection rate, 44%, could be due to climate differences between Iraq and Iran plus differences between the hygienic systems between the two countries. Moreover, the detection methods could also add variations in the results.

E. histolytica causes amoebiasis which is an important parasitic disease that infects millions of people with thousands of mortalities every year. The current work identified the presence of this protozoan in the fecal samples of cattle from Babylon province, Iraq. Those findings agree with those obtained by (11–16) who identified, especially in countries with poor health systems, different infection sources for the disease occurrence; however, public water contaminated play as important sources in the transmission of the disease.

The present study results showed the identification of C. parvum in the tested fecal samples of cattle from Babylon Province, Iraq, and those findings agree with those recognized by 17 who provided information about the importance of cattle as an infection source

for humans and other animals with inducing a diarrheal disease in humans and loss of production in the infected animals.

Conclusion

The data from the current investigation identify the presence of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in the fecal samples collected from cattle located in Babylon province, Iraq.

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Conflict of interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Branch of Parasitology, Collage of Veterinary Medicine, University of Al-Qasim Green and all experiments were carried out in accordance with approved guidelines.

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