

Molecular Epidemiological Studies Of Giardiasis In Cattle

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Abstract

We conducted the molecular epidemiological study of cattle in Lahore from August 2007 to July 2008. Faecal samples were collected from four different areas, i.e. Military dairy farm, Government dairy farm, Gawala dairy colonies and House hold dairy animals. The faecal samples were purified and the number of cysts purified and quantified. DNA was extracted from the purified cyst and was amplified through PCR. The study revealed the prevalence 31.11 % (224/720), amongst these the high prevalence was recorded in Govt. dairy farm (46.11%), followed by Gawala dairy colonies (36.11%), Military dairy farm (25.55%) and lowest was recorded in household dairy animals (16.66%). The age-specific herd prevalence was (41.66%) and (27.59%) young and older cattle, accordingly. The high prevalence was observed in female (31.71%) as compare to the male (26.25%). The season wise prevalence was recorded higher in autumn (36.66%) followed by summer (34.58%), spring (30%) and lowest was recorded in winter (25%). The statistical analysis revealed the higher significant in all areas and also in age, sex and season wise ($p < .05$). The cyst excretion levels varied considerably both within and between herds for all age of area. It was noted that the monthly distribution pattern of Giardiasis in bovine was related to the temperature, humidity and the rainfall.

INTRODUCTION

Giardia lamblia, also called *Giardia duodenalis* or *Giardia intestinalis*, is a protozoan parasite of the small intestine that causes extensive morbidity worldwide. *Giardia* was organism seen under the microscope and described by Antony Van Leeuwenhock in Nov. 1681 (Dobel 1920). It was rediscovered and described by Vilim Lambli in 1859. The genus name *Giardia* was proposed in 1882 and *Lambli* in 1988. Most workers in this field use the name *Giardia* (Meyer 1990, 1994) and research into its epidemiology, pathogenesis, and treatment has intensified since *G. lamblia* waterborne outbreaks were reported in Europe and the United States during the 1960s and 1970s (Craun, 1990; Jephcott et al, 1986; Farthing 1992). *Giardia Duodenalis* is the most commonly reported intestinal parasite of human in developed country (Thompson et al 1990, Shantz, 1991). *G. duodenalis* is capable of infecting a wide variety of mammalian hosts as well as birds, reptiles and amphibians (Thompson et al 1993, Kulda and Nohynkova, 1995). Aquatic animals have been described in the contamination of water sources with *Giardia* cysts (Davis and Hibler, 1989., Dykes et al 1980, Erlandsen et al 1990). *Giardia* infection have been reported in various species of farm animals (Xiao, 1994, Olson et al 1997). Domestic animals can be a source of surface water contamination by pasture runoff (Weniger et al 1983, Craun, 1990). Dairy calves can excrete high number of

the cyst of *Giardia* and the disease in cattle is clinically important that can reduce the growth performance of the ruminants (Olson et al 1995, and Ralston et al 2003).

Diagnosis of *Giardia* by the conventional microscopic methods following of fecal concentration techniques, particularly zinc sulphate and zinc chloride floatation and centrifugation (Zajac et al, 2002) remained a reliable indicator of the infection but however there is a need for sensitive and specific diagnostic method for detection the etiological agents of the disease, with reference to *Giardia*, molecular techniques such as PCR provide alternate methods for specific detection of the pathogens in stool. The sensitivity of detection by PCR is greater than that of microscopy, making it of great use for detection of low number of parasites in stool samples (McGlade et al. 2003). The PCR-based procedures have greater sensitivity and specificity than the conventional diagnosis that are reliant on microscopy. So far no study has been conducted in our country regarding molecular diagnosis of Giardiasis in cattle. Keeping in view its high prevalence in bovines the present study is designed to carry out the epidemiological study base on PCR.

MATERIALS AND METHODS

The cattle's of different ages of the Government dairy farm, Military dairy farm, gawalla colonies and house hold dairies

in Lahore were randomly selected and studied for the prevalence of Giardiasis.

A total of 720 samples were studied through out the year and 180 samples in each area's were studied. Similarly 15 samples were studied in week of each area i.e Military dairy farm, Govt.dairy farm, gawalla dairy colonies and Household dairy in Lahore. The samplings were collected from august 2007 till the end of July 2007.

SOURCES & COLLECTION OF SPECIMEN

A total of 720 fecal samples were collected randomly from the cattle's located in the government dairy farm, military dairy farm, Gawala colonies and house hold dairy in Lahore during the period August 2007 to July 2008. The feces were collected directly from the rectum using disposables gloves for every animal. The samples were put into individual clean plastic bags, the bags were sealed and labeled the animals tag no., date and form/areas of the collection. The samples were stored in cooler in ice and immediately transported to the postgraduate laboratories of parasitology, university of veterinary and animal sciences Lahore.

ISOLATION OF CYST

Giardia cysts were isolated from the fecal samples as the method described by O, Handley et al (1999). The procedure was modified according to the cattle to accommodate the fecals size of 20 grams. The fecal samples were mixed in 35 ml of phosphate buffered saline in Petri dish, Thoroughly mix and the slurry was filtered three time through cheesecloth and pour them into the 50ml centrifuge tube and layer over 15ml of sucrose (specific gravity 1.13) .The samples were centrifuged at 800 x g for 5 minutes .The upper layer and the interface layer were transferred through the pipette into other clean centrifuge tube and add the equal amount of phosphate buffered saline and recentrifuged at 800 x g for 5 minutes. The supernatant was discarded and the pellet was resuspended with PBS to a final volume of 1 ml in clean ependroff.

DNA EXTRACTION

The fecal samples resuspended were subjected to DNA extraction by the method of da saliva et al (1999) with slight modification. The tissue DNA extraction kit (GFC) Vivantis was used throughout the experiment. The ependroff were weighed by electric balance and average 0.97gm.then added the resuspended fecal material and filled in half of the ependroff and again weighs i.e. average 1.32gms of the each sample. After this, samples were properly numbered and

added the 200ul TL buffer, 5 ul proteinase K and 200ul cell lysis enhancer from the tissue DNA extraction kit and incubate the ependroff at 37° c for 24 hours., after incubation added the 200ul TB buffer mix thoroughly and place in water bath at 65°c for 20 minutes, after this process the samples were centrifuged at 13200 r pm for 30 minutes and the supernatant was collected in clean molecular grade ependroff and further added the 200ul absolute ethanol mix immediately and centrifuge at 13200 rpm for 35 minutes. The pellet was resuspended 750ul wash buffer and centrifuge at 13200rpm for 30 minutes. The supernatant was discarded and pellet wash 2-3 times until clear pellet of DNA was obtained .The pellet was added in TBE buffer and store at -80 °c.

The confirmation of the pellet DNA was made through electrophoresis. The samples were loaded in the wells of 1% agarose gel.5ul bromophenol blue dye mix with 10ul DNA sample and similarly 15ul sample loaded in each well of the gel. Than supplied the current at 100 volt and run for 40 minutes. The gel transfer to gel DOC and confirmed DNA samples were preserved at -80°c for amplification.

AMPLIFICATION OF DNA (PCR) AND DETECTION

753 bp products of Giardia primers were amplified. The forward sequence of primers was G-7 (5AAGCCGACGACCTCACCCGAGTGC-3) and synth ID No. 265257 and reverse primers sequence was -G759 (5-GAGGCCGCCCTGGATCTTCGAGACGA-3) and synth ID No. 265259.The PCR mixture was consist of 1x buffer having 1.5mM Mgcl2, 200uM dNTP,s, 25pmol of each primers, 1.25U of Tag polymerase enzyme and 5ul of purified DNA in final volume of 50ul. PCR was run with initial temperature at 94oC and then set the programmed file at T=65°c and G=10 and run for 35 cycle. It completed in two hours. Than thermocycler regulates to 4°c than PCR tube was taken out and the product was detected by 1% Agarose gel electrophrosis and visualized by ethidium bromide staining.

PREVALENCE RATE

The prevalence rate of disease, Giardiasis amongst the cow, buffaloes and calves (male female) were determined in light of the following formula of threshold et al 1997.

$$\% = \left(\frac{\text{No. of disease animal}}{\text{Total no. of animal present.}} \right) \times 100$$

Similarly the month wise, age wise, sex wise and season

wise prevalence of disease was also determined.

METEOROLOGICAL DATA

The meteorological data regarding humidity, temperature, cold and rainfall were collected from the meteorological department of Lahore on weekly basis and its effect on disease prevalence was determined.

RESULTS

A total of 720 fecal samples were treated with GF-1 tissue DNA extraction kit (Vivantis), amongst these 224 DNA were amplified through PCR of the cattle in different area's / farms in Lahore. So the molecular prevalence rate was 31.11% (224 /720) in cattle. Statistically significant higher ($P<0.05$) in area, wise as well as months wise in prevalence.

1. Area wise.

Area wise molecular prevalence (%) was shown highest in Government dairy farm (46.11%) followed by Gawala colonies (36.11%), Military dairy farm (25.55%) and lowest in House hold dairies (16.66%). Statistical analysis by chi square test, prevalence was significantly higher ($P<0.05$).

2. Season wise.

Season wise molecular prevalence (%) was noted highest in autumn (36.66%) followed by summer (34.58%), spring (30.83%) and lowest in winter (25%). Statistical analysis by chi square test, significantly higher ($P<0.05$).

3. Age wise.

When the data was analyzed on the basis of group it was noted that the cattle shows high prevalence in young (41.66%) of age 2-3 years as compare the cattle having age 3-7 years (27.59 %). Statistically it was higher ($P<0.05$).

4. Sex wise.

In sex wise data female showed slightly higher (31.71) prevalence than male (26.25) with statistically ($P<0.05$).

5. Association between Giardiasis and Meteorological Factors

It was observed that the highest prevalence of Giardiasis in cattle (40 %) was noted in August when the average temperature was 31.48°C. However the maximum and minimum temperature was 35.37°C and 27.6°C respectively. The average relative humidity was 71.28% where its range was 66.38-76.19% and rainfall was noted 3.2mm. It was

observed that the monthly pattern of distribution of Giardiasis was greatly concern with the temperature particular the high % of humidity as compare to the other months of the year and also the rainfall.

DISCUSSION

In this study fecal samples were obtained from bovine over in 12 months of period from August 2007- July 2008, from four different areas. This is the first long study in Pakistan particularly at Lahore to report prevalence, microscopic diagnosis and molecular characterization data of *Giardia* spp in bovine from military dairy farm, Gawala colonies, government dairy farm and house hold dairy. The present study showed some epidemiological aspects of Giardiasis in bovine. The occurrence of Giardiasis in military dairy farm, Gawala colonies, government dairy farm and household dairy is influenced by multifactor system that is composed of host, parasitic agents, transmission process and environmental effects.

Most previous study on *Giardia* in human and bovine were based on the microscopy examination, others methods for detection of the cyst in stool specimen like enzyme immunoassay for stool antigen and *Giardia* CELISA (CEUABAS pty Ltd). These assays are not enough to detect low levels of infection (Johnston et al 2003). In the present study, polymerase chain reaction (PCR) diagnosis was higher than microscopic. The PCR based prevalence was recorded 31.11 % (224/720) in cattle the statistical analysis shows the significant difference was ($P<0.05$). The molecular study shows more significant value of P than the microscopic. The sensitivity of detection by PCR is greater than that of microscopy, making it of great use for detection of low numbers of parasites in stool samples (McGlade et al 2003).

Considering the role of the meteorological data in the spread of Giardiasis in cattle it was noted that the monthly distribution pattern of Giardiasis in bovine was related to the temperature, humidity and the rainfall. Gradual increase in average temperature 19.98-30.09°C associated with increased and rainfall. . High prevalence of Giardiasis was noted in August and October(40%) and (38.33%). The temperature also increased up to 31.48°C with increase of humidity 71.28% and rainfall 3.2mm. After rainfall, humidity was increased in August. It was noted that with increase of temperature and humidity, there is increase of % prevalence of Giardiasis in cattle. It the months of November and January, where temperature is very low i.e. 20.41°C and

14.66°C and the humidity was noted 67.09% and 65.32%. The prevalence of Giardiasis in cattle was also noted very low. It shows the temperature and humidity play major role in its prevalence, Similar finding reported that the Giardia infection were more frequently diagnosed in rainy months of August and September in diarrhetic patient of Bangladesh (Alam et al personal communication) and in human Giardiasis in Saudi Arabia in months of September was higher prevalence (16%) (Kasim and Elhelu, 1983).

Figure 1

Fig;1

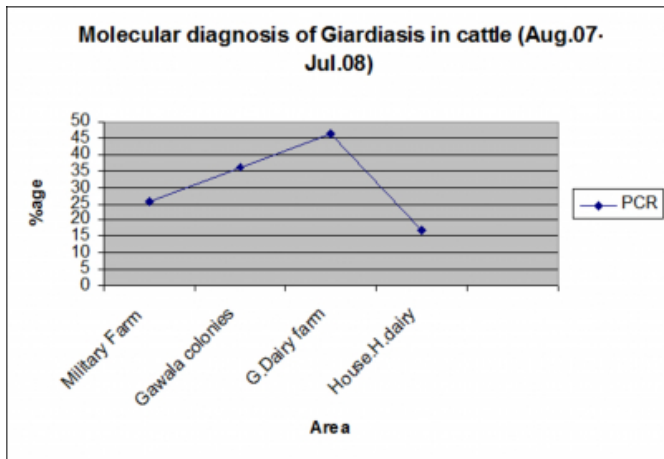


Figure 2

Table. No.1 MOLECULAR DIAGNOSIS OF GIARDIASIS IN CATTLE (Aug.07-Jul.08).

Factors		No. of positive/ Total samples	Prevalence (%)
Area.	Military dairy Farm	46/180	25.55%
	Gawala Colonies	65/180	36.11%
	Govt.dairy Farm	83/180	46.11%
	House hold dairies.	30/180	16.66%
Season	Spring	37/120	30.83%
	Summer	83/240	34.58%
	Autumn	44/120	36.66%
	Winter	60/240	25%
Age	2-3 years	75/180	41.66%
	3-7 years	149/540	27.59%
Sex	male	21/80	26.25%
	female	203/640	31.71%

Statistical analysis. Chi square test. Significant $P < 0.05$, non-significant $P > 0.05$.

Figure 3

Table No. 2 Mean Monthly Temperature, Humidity and Rain Fall in Lahore During 2007-2008

Time (Months)	Temperature (C°)			Relative Humidity (%)			Rainfall (mm)
	Minimum	Maximum	Mean	Morning	Evening	Mean	
August 2007	27.6	35.37	31.48	76.19	66.38	71.28	3.2
September 2007	25.4	33.85	29.62	75.9	59.6	67.3	2.52
October 2007	19.20	32.50	25.85	67.37	85.08	76.22	0.00
November 2007	14.44	26.39	20.41	81.23	52.96	67.09	0.34
December 2007	08.50	20.83	14.66	81.20	49.45	65.32	0.106
January 2008	06.09	17.80	11.94	76.29	46.32	61.30	0.78
February 2008	09.40	21.17	15.28	72.65	47.53	60.09	0.19
March 2008	19.10	30.90	25	67.30	36.80	52.05	0.00
April 2008	28.38	33.30	30.84	53.13	33.10	43.11	1.93
May 2008	25.40	37.70	31.55	47.36	31.70	39.53	1.18
June 2008	27.15	35.82	31.48	72.34	55.20	63.77	2.38
July 2008	28.36	35.49	31.92	77.35	62.48	69.91	3.78
Mean	19.98	30.09	25.03	70.77	52.46	61.61	1.45

Figure 4

Fig A. Gel showing DNA of Giardia

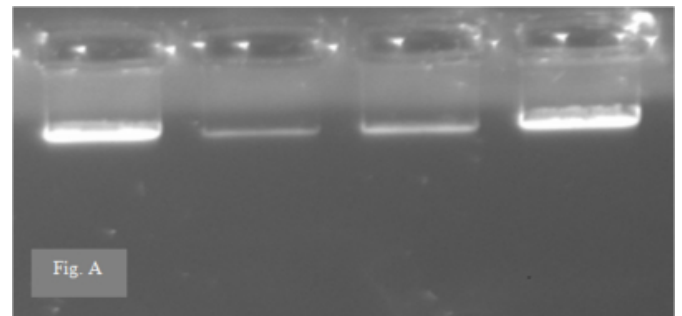
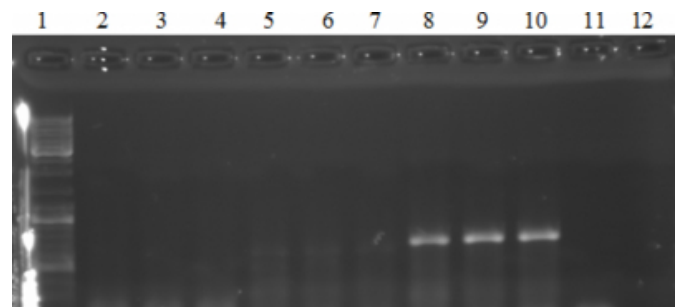


Figure 5

Figure B. Giardia DNA amplification by PCR / Lane 1: 2 KB Ladder Marker / Lane 2-7, 11 & 12: Negative samples / Lane 8-10: Positive sample (753bp product)



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