SHORT REPORT
Intestinal parasitism and *Vibrio cholerae* infection among diarrhoeal patients in Kolkata, India

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SUMMARY

In this study, we have analysed the epidemiological significance of the concurrent infections caused by *Vibrio cholerae* and intestinal parasites among different age groups of hospitalized diarrhoeal patients in Kolkata. A total of 3556 stool samples collected during 1996–2004 were screened for vibrios and parasites. The seasonality of *V. cholerae* and parasitic infections were studied in detail. The detection rates for *Ascaris lumbricoides* and *Giardia lamblia* infection were more than for the hookworm, *Trichuris trichiura* and *Entamoeba histolytica*. *V. cholerae* O1 was identified as the predominant serogroup among diarrhoeal patients. The highest rates for *V. cholerae* infection were in the 2–5 years age group and combined infection of *V. cholerae* and parasites was recorded among children aged between 2 and 10 years.

Toxigenic *Vibrio cholerae is* a Gram-negative enteric pathogen that causes sporadic, epidemic and pandemic cholera. Many of the cholera pandemics originated in the Ganges valley of the Indo-Bangladesh subcontinent, which is commonly known as the 'homeland of cholera'. Parasitic infestations are one of the important aetiological agents of diarrhoea, affecting over half of the world's population on a chronic or recurrent basis, often associated with significant morbidity [1]. Gastrointestinal helminthic infection is high in the developing countries [2]. Malnutrition, poor absorption, diarrhoea and anaemia are frequently encountered in these patients. High incidence of intestinal parasitism has been reported from eastern parts of India (West Bengal) with a mixed parasitic infestation rate of about 60–80% of the population [3]. A rural community-based study from Kolkata, recorded about 66·4% of preschool

children as suffering from gastrointestinal complaints with worm infestation [4]. Similarly to intestinal helminthes and malaria [5], the overlapping infection of intestinal parasites and cholera may result in a higher rate of morbidity. This study aimed to discover the level of concurrent infection caused by parasites and *V. cholerae* among different age groups of hospitalized diarrhoea patients and the seasonal trends.

This study is part of the hospital-based active surveillance programme of National Institute of Cholera and Enteric Diseases, Kolkata. Only patients with acute diarrhoeal illness admitted in the Infectious Diseases Hospital were considered in this study. A total of 3556 stool specimens collected during the period 1996–2004 were included in the study and the patients were selected irrespective of age and sex. Stool specimens (not rectal swabs) were processed for isolation of *V. cholerae* following standard methods [6].

For identification of different parasites, a uniform thin suspension of stool was made with one or two drops of normal saline and 1% Lugol's iodine in

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Table. *Distribution of cholera cases, by age, with or without intestinal parasitism among diarrhoeal patients*

| | Infection status | | | | | | |
| | Diarrhoea | | Parasite and | | | | |
Age (years)	cases	*V. cholerae*	*V. cholerae*	Parasite	OR	95% CI	*P* value
<2	561	91 (16·2)	16 (2·9)	26 (4·6)	2·89	1·42–5·88	0·001
2–5	280	106 (37·9)	43 (15·4)	27 (9·6)	1·56	0·87–2·82	0·112
>5–10	186	40 (21·5)	28 (15·1)	39 (21·0)	1·42	0·73–2·75	0·267
>10–20	535	83 (15·5)	32 (6·0)	118 (22·1)	0·99	0·61–1·60	0·955
>20	1994	265 (13·2)	46 (2·3)	334 (16·8)	0·70	1·01–2·02	0·037

OR, Odds ratio; CI, confidence interval.
Figures in parentheses are percentages.

two separate glass slides, each was then covered with a 22 mm² coverslip. The entire film was examined for the presence of helminthic ova/larvae and protozoan cysts and trophozoites. The negative stool specimens for ova and cysts, were subsequently examined for the presence of intestinal helminth parasites by the formalin ethyl acetate concentration method [7]. A data entry form designed to run within the Epi 2002 software program provided by the CDC (Atlanta, GA, USA) was used in this study. The validated data were compiled with SPSS 13.0 (SPSS Inc., Chicago, IL, USA) for preliminary analysis. The Mantel–Haenszel χ^2 test was employed to compare the infection difference between *V. cholerae* and parasites and the odds ratio was explored for all age groups.

The Table shows distribution of parasitic infestation by age among diarrhoeal patients with or without *V. cholerae*. Comparative analysis, according to age, has shown that *V. cholerae* infection was high among the 2–5 years age group (37·9%). On the other hand, the 2–5 and >5–10 years age groups had *V. cholerae* with parasitic infection at the rate of 15·4% and 15·1%, respectively. Among children aged <2 years, 2·9% had concurrent infection with parasites and *V. cholerae*, and 4·6% of them had *parasitic* infection alone (OR 2·89, 95% CI 1·42–5·88, *P* < 0·001). This trend was not seen in patients in other age groups.

Seasonal occurrence of parasites (Fig. 1) demonstrated high infection of *Ascaris lumbricoides* throughout the year with maximum isolation in summer (May and June). *Trichuris trichiura* infection was much lower than *A. lumbricoides*. Regarding hookworms, infection was also highest in summer (March–June). Occurrence of *Giardia lamblia* infection was greater in summer (March–April) and
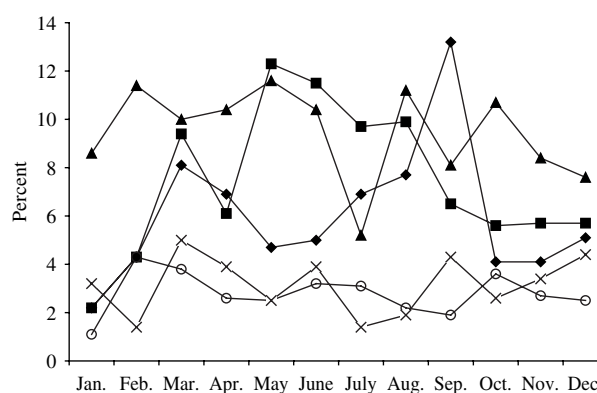

Fig. 1. Seasonality of parasitic infections among hospitalized diarrhoeal patients in Kolkata during 1996–2004. ◆, *Giardia lamblia*; ■, *Entamoeba histolytica*; ▲, *Ascaris lumbricoides*; ○, *Trichuris trichiura*; ×, hookworm.

during the monsoon (July–August) with a peak in September. *Entamoeba histolytica* infection appeared to be uniformly present throughout the years with a peak during summer and monsoon seasons (May–August). From the yearly seasonal variations (Fig. 2), it appears that starting in April, cholera infection continued until December with a peak in September and October. Among the different serogroups, *V. cholerae* O1 was the predominant serogroup in this study.

This study confirms that the occurrence of parasitic infection is more frequent in *V. cholerae*-infected patients in the <2 years age group (almost three times higher). Few studies report the concurrent prevalence of parasites and *V. cholerae* among diarrhoeal patients [8–10]. To our knowledge, this is the first extensive report on the occurrence of cholera associated with intestinal parasites among diarrhoeal patients in different age groups. It has been reported
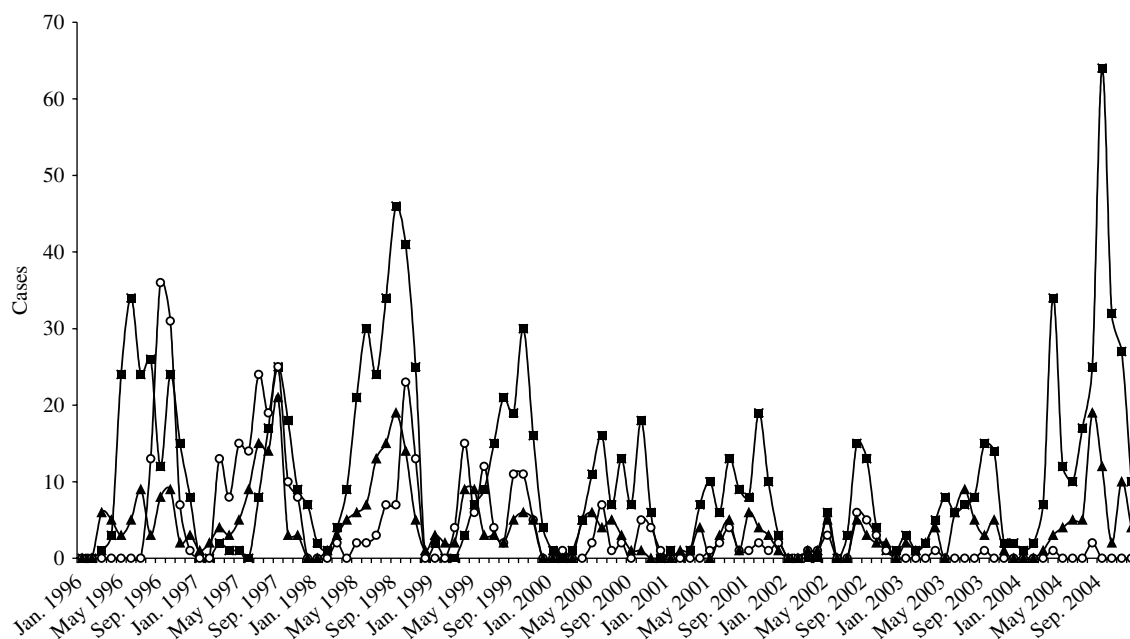
Fig. 2. Seasonality of different serogroups of *V. cholerae* among hospitalized diarrhoeal patients during 1996–2004. ■, Serogroup O1; ○, serogroup O139; ▲, non-O1, non-O139 serogroups.

that helminthic infections may alter the immune response to non-parasitic antigens in experimental animal models [11] and also suppress the action of cholera vaccine [12]. Impaired digestion, malabsorption, reduced food intake and diarrhoea are frequently encountered in parasitic infections, especially in children suffering from ascariasis and trichuriasis, amoebiasis and giardiasis. The clinical illness may be aggravated if the parasite-infested children are simultaneously infected with *V. cholerae*.

Earlier reports have documented that cholera peaks in Kolkata during April–June [13]. Our study results showed the maximum number of cholera cases during August–October. This aspect should be studied in greater detail, correlating the incidence of cholera with many factors such as rainfall, humidity, temperature, along with sanitation and quality of water at consumption points. In addition, basic education, health consciousness, better nutrition and personal hygiene are urgently needed to reduce the rate of both cholera and parasitic infections.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Chan MS.** The global burden of intestinal nematode infections: fifty years on. *Parasitology Today* 1997; **13**: 438–443.
2. **Bundy DAP.** Immunoepidemiology of intestinal helminthic infections. 1. The global burden of intestinal nematode disease. *Transactions of Royal Society of Tropical Medicine and Hygiene* 1994; **88**: 259–261.
3. **Chakrovorty AK,** *et al.* Health status of rural population of Singur as revealed in repeat general health survey. *Indian Journal of Medical Research* 1978; **68**: 1018–1028.
4. **Saha DR,** *et al.* Intestinal parasitism: a childhood problem in rural Bengal. *Journal of Communicable Diseases* 1995; **27**: 170–174.
5. **Nacher M,** *et al.* Malaria vaccine trials in a wormy world. *Trends in Parasitology* 2001; **17**: 563–565.
6. **WHO.** Manual for laboratory identification of acute enteric infections, CDD/83-3/Rev. 1987-113, Geneva, Switzerland: World Health Organization, 1987.
7. **WHO.** *Basic Laboratory Methods in Medical Parasitology*, Geneva, Switzerland: World Health Organization, 1991.
8. **Faulkner CT,** *et al.* Prevalence of endoparasitic infection in children and its relation with cholera prevention

efforts in Mexico. *Pan American Journal of Public Health* 2003; **14**: 31–41.

9. **Tamang MD, *et al.*** An outbreak of El Tor cholera in Kavre district, Nepal. *Kathmandu University Medical Journal* 2005; **3**: 138–142.

10. **Nagoba BS, *et al.*** Association of *Giardia lamblia* with *Vibrio* in cholera cases. *Indian Journal of Pediatrics* 1992; **59**: 386–387.

11. **Actor JK, *et al.*** Helminthic infection results in decreased virus-specific CD 8 cytotoxic virus-specific T-cell and Th-1 cytokine responses as well as delayed virus clearance. *Procedings of National Academy of Sciences USA* 1993; **90**: 948–952.

12. **Cooper PJ, *et al.*** Human infection with *Ascaris lumbricoides* is associated with suppression of the interleukin-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. *Infection and Immunity* 2001; **69**: 1574–1580.

13. **Kaper JB, Morris Jr. JG, Levine MM.** Cholera. *Clinical Microbiology Reviews* 1995; **8**: 48–86.