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Original article

Prevalence and zoonotic potential of intestinal protozoans in bovines in Northern India

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ABSTRACT

Bovines, and especially cattle, have a dual position of appreciation in India, being both important in the food industry as providers of dairy products, and, culturally, being considered as holy creatures that must not be harmed, killed or eaten. This status means that cattle have a paradoxical existence in India; as they are worshipped and protected, they are able to roam freely among humans, but they are also often left to fend for themselves. The water buffalo represents a significant contributor to the Indian agricultural economy as well as general social development, and are in this way somehow replacing the indigenous cattle. The vast numbers of roaming cattle without clear owners are difficult to look after in terms of veterinary healthcare and appropriate interventions when necessary, and have no regular supply of food.

This article describes an investigation of the occurrence of *Cryptosporidium* spp. and *Giardia duodenalis* in bovines either roaming the streets or being kept in animal holdings in and around Chandigarh, a city in Northern India, and addresses the zoonotic potential of these protozoan parasites shed from bovines living in close contact with humans. 294 animals of all ages were sampled, and the majority of the positive samples were found from calves. The overall prevalence of *Giardia* was 8.2% and *Cryptosporidium* was 2.4%. Non-zoonotic assemblages were predominantly found in the case of the *Giardia* – positive samples, and in the case of *Cryptosporidium*, as well as non-zoonotic genotypes, zoonotic subgroups previously described from infected human infections in this area, were identified, indicating that there may be sharing of intestinal parasites in these settings, where cattle live in close contact with humans.

1. Introduction

Cryptosporidium and *Giardia* are two of the most common agents of infectious enteritis in humans and animals worldwide. From a public health perspective, it is imperative to understand the sources and routes of transmission in different geographical regions. *Cryptosporidium* and *Giardia* infections are known to cause production losses in bovines (Olson et al., 1997), and are considered potential sources of human infection as well, with pre-weaned calves and lambs recognised as important reservoirs of the zoonotic *C. parvum* in some countries (Santin-Duran and Trout, 2008).

The extent and relative importance of zoonotic transmission of these parasites in different parts of the world, especially in the developing countries, are still poorly understood (Abeywardena et al., 2015). Although for *Giardia*, it is generally considered that most human infections are from direct or indirect human-to-human transmission, rather than zoonotic (Monis and Thompson, 2003), for *Cryptosporidium*, a different picture occurs.

In human populations, it is generally considered that *Cryptosporidium parvum* tends to dominate in Europe, New Zealand, and the Middle East, indicating a potential for zoonotic transmission, whereas *C. hominis* are responsible for more human infections than *C. parvum* in USA, China, Japan, and the majority of developing countries (Cacciò and Putignani, 2014).

Indeed, studies from India tend to support this distribution; three studies from Northern India indicated that over 70% of *Cryptosporidium* infections were caused by *C. hominis* (Yadav et al., 2017; Gatei et al., 2007; Sharma et al., 2013), which is in concordance with the general

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finding of Xiao and Fayer (2008), who reported that, in the studies where molecular methods have been used, *C. hominis* has, in general, been associated with a higher number of human infections than *C. parvum*.

However, despite this distribution, the role of cattle in transmission or propagation of these parasites in India is of particular interest, due to the particular interactions between bovids and humans here. India is home to a quarter of the world's cattle population. In Hindu religious scriptures, the cow is referred to as the "all-producing and all-containing universe" (Korom, 2000), and the cow is the most sacred of all animals according to the Hindu religion. Northern India practices a total ban on cow slaughter, and the Indian Veterinary Council has estimated that there is only sufficient food to sustain 60% of the Indian cattle population, and the rest are left to starve or roam the streets searching for food, which, in most cases, ends up being a diet of garbage. In New Delhi, there is an estimated number of 40,000 stray cattle, and in Chandigarh this number has risen from 1400 to 2000 during the last five years (Agoramoorthy and Hsu, 2012; Victor, 2013; Kang, 2003). This creates an obvious difference in animal management from industrialized countries, where cattle are kept on enclosed farms and restrictions between animal and human contact are mandatory by law due to zoonosis and hygiene considerations. The situation in India also differs from that in other developing countries, where cow ownership and cattle as a staple means of livelihood means that cattle are handled in such a way to as to ensure that their productivity as dairy or beef animals is maximised, and unrestricted wandering, particularly in urban settings, is relatively uncommon. The special role of cows in Indian culture means that there is an unusual interface between humans and animals, and the sources of infection, for both human and animals, are likely to be strolling along, or sometimes scavenging for food, in the same streets.

In this study, faecal samples from cattle and water buffaloes in urban and peri-urban areas of Northern India were collected, and the prevalence and zoonotic potential of *Cryptosporidium* and *Giardia* were assessed.

2. Material and methods

2.1. Sampling

From March 2014 until February 2016, 294 samples were collected from different animal holdings as well as stray animals in and around Chandigarh, Northern India (Fig. 1). Of these, 109 samples were collected during the winter and spring season (from mid-September to March), and 185 during the monsoon season (from mid-July to mid-September). 83 of these samples were from water buffaloes and 7 were sampled from stray cattle. With respect to where the various bovines lived, 153 samples were collected from animal holdings within the city (urban), and 141 from animal holdings and settlements surrounding the city (peri-urban). Only one sample was collected per animal per sampling occasion. The ages of the animals ranged from calves under three months to adults. Presence of diarrhoea in samples and approximate age of animal was recorded at sampling. The samples (each approximately 5–10 g) were collected either rectally or non-invasively promptly after defecation, and were immediately mixed with 2.5% potassium dichromate and stored at 4 °C before transportation to the Parasitology Department, Norwegian University of Life Sciences (NMBU) for analysis.

2.2. Analysis for occurrence

From each sample collected, 3 g of faeces were homogenized with 57 ml water and passed through a faecal parasite concentrator with a pore diameter 425 µm (Midi Parasep, Apacor, Berkshire, England). The suspension was transferred to 10 ml centrifuge tubes and centrifuged at 1550 rfg for 3 min to create a pellet. The supernatant was discarded, and between 5 and 20 µl of homogenized and sieved faecal material was placed on a microscope slide using plastic bacteriological loops that take approx. 10 µl amount of sample. The samples were left to dry and then fixed with methanol before staining with 15 µl of monoclonal antibody for *Cryptosporidium* oocysts and *Giardia* cysts (Aqua-Glo G/C, Waterborne, Inc., New Orleans, L.A), and then incubated at 37 °C in a humid chamber for 45 min. The staining solution was then rinsed off with distilled water, and a coverslip placed over the sample before immediate microscopic examination (Fig. 1).

Prepared samples were screened under a fluorescence microscope with the following filter settings: FITC: emission- 490 nm, excitation – 525 nm.

The samples were graded after counting the number of cysts/oocysts per field of view at ×20 objective magnification:

2.3. DNA isolation

For *Giardia/Cryptosporidium*-positive samples, DNA was isolated from 200 µg of concentrated faeces using the QIAmp DNA mini kit (Qiagen GmbH). The protocols followed the manufacturers` instructions with slight modifications; cysts/oocysts were first mixed with 150 µl of TE buffer (100 mM Tris and 100 mM EDTA) and incubated at

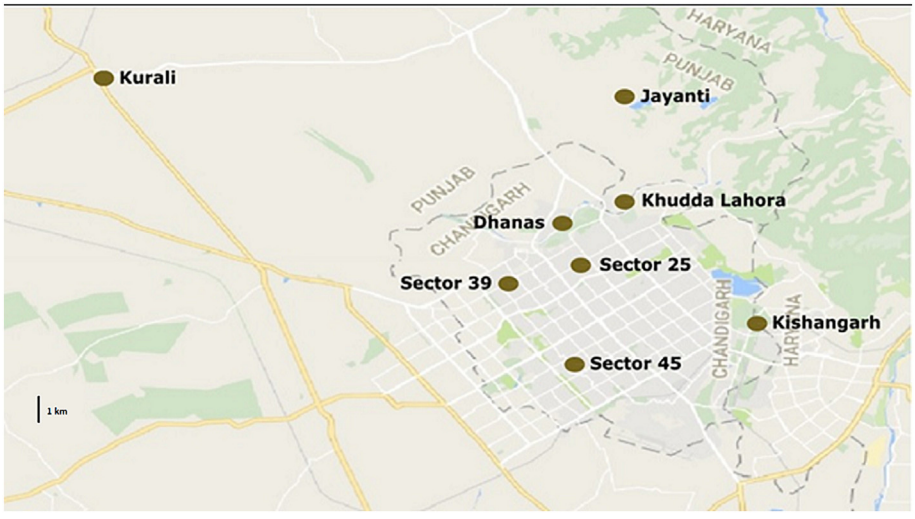

Fig. 1. Map of sampling sites.