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Changes in the seroprevalence of cysticercosis in suspected patients in Chandigarh, India between 1998 and 2014: analysis of 17 years of data

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SUMMARY

Changes in seroprevalence of cysticercosis diagnosed in Chandigarh, India between 1998 and 2014 were investigated by extraction and analysis of data from records held at the Postgraduate Institute of Medical Education and Research in Chandigarh. Among the total number of samples for which cysticercosis had been suspected during this period ($N = 9650$), 1716 (17·8%) were seropositive. Adults were more likely to be seropositive than children, and women were more likely to be seropositive than men. In addition to there being fewer patients with suspicion of cysticercosis over the data analysis period, the proportion of patients seropositive also reduced significantly. Despite these reductions, which are probably associated with improved infrastructure and sanitation within Chandigarh, and despite meat consumption being relatively rare in this area, the extent of cysticercosis in this population remains problematic. Further efforts should be made to reduce transmission of this infection, with particular emphasis on women. Such efforts should follow the One Health concept, and involve medical efforts (including diagnosis and treatment of *T. solium* tapeworm carriers), veterinary efforts directed towards meat inspection and prevention of infection of pigs, and environmental health and sanitation engineers (to minimize environmental contamination with human waste).

Key words: Analysis of data, control, cysticercosis, foodborne zoonoses, taeniasis.

INTRODUCTION

The life-cycle of *Taenia solium* involves human infection with the adult tapeworm, which resides in the small intestine, and larval stages (Cysticercus cellulosae) in pigs. Humans become infected with the adult tapeworm (taeniosis) by consumption of undercooked pork containing viable larval stages, and pigs are infected by consumption of the tapeworm eggs that are excreted in the faeces of the infected person. An adult tapeworm can contain around 800 proglottids, each of which contains 30 000-40 000 eggs, so the potential for environmental contamination and onwards transmission to the pig intermediate host is great. Human cysticercosis is caused by the presence of *T. solium* larval stages; this may be due to ingestion of *T. solium* eggs or, less frequently, from reverse peristalsis in a person infected with the adult *T. solium*.

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The pathology of cysticercosis is due to the development of cysticerci in different sites in the body, and may include subcutaneous, intramuscular, cardiac, spinal, ocular, and CNS locations. The latter is of greatest concern; neurocysticercosis (NCC) can be fatal and the symptoms can include seizures, mental disturbances, and focal neurological deficits.

Ingestion of *T. solium* eggs may occur directly by hand-to-mouth and is particularly relevant for someone who is themself infected with the adult tapeworm, or there is someone in their household who is infected. However, a vehicle of infection, such as contaminated food or water may also be responsible. Due to the serious nature of NCC and its widespread prevalence in many regions of the world, *Taenia solium* has been repeatedly recognized as the most important foodborne parasite [1, 2], with foodborne cysticercosis resulting in the highest burden globally among foodborne parasitoses, as measured in disability adjusted life years (DALYs) [3].

Given that the life-cycle of *T. solium* is reliant on the consumption of undercooked pig meat, and that meat consumption in India is among the lowest (kg/*per capita*) globally [4], it might be expected that cysticercosis would be relatively rare in India. However, the huge production of eggs in a single *T. solium* infection, coupled with problems associated with sanitation infrastructure, means that even relatively few cases of taeniosis can result in considerable environmental contamination and the possibility of transmission, both to pigs, thereby completing the life-cycle, and also to humans, resulting in cysticercosis. It should be noted that in India, pig rearing is generally associated with lower socioeconomic groups, with pigs being free-ranging and often slaughtered at home or in butcher's shops [5]. This means that there is easy access to human faeces, and meat inspection is minimal or non-existent.

Although much of the published data from India on cysticercosis and NCC are case reports, there are some more comprehensive articles that consider the burden and prevalence of these conditions. For example, a study in the epilepsy population of Jammu and Kashmir, Northwest India provided a point prevalence of of 4·5/1000 for NCC and the authors concluded that a high prevalence of NCC was a major reason for symptomatic epilepsy in that region [6]. A comparable study from Dehradun in Uttarakhand in Northern India provided similar data [7]. In Vellore, South India, a similar study provided slightly lower results, with a point prevalence for NCC in their population being 1·28/1000 in urban areas and 1·02/1000 in rural areas, and the authors concluded that NCC was a major contributor to active epilepsy here also [8]. Furthermore, a study that considered the burden from NCC in India during 2011 [5], found that NCC caused significant health and economic impacts, with the annual health burden per thousand persons calculated as 1·73 DALYs and an annual economic burden of 12·03 billion rupees (>€160 million) [5].

However, initiatives are in place in India that should impact on a range of neglected tropical diseases and diseases of poverty, not least of which are 'Nirmal Bharat Abhiyan' (also known as the Total Sanitation Campaign), which ran from 1999 to 2014 and was a people-centred sanitation campaign with the goal of eliminating open defecation, and 'Swachh Bharat Abhiyan' (Clean India Mission), which was launched in 2014 with the purpose of cleaning India in general, with particular emphasis on infrastructure. Thus, it is hoped that the prevalence and impact of cysticercosis and NCC, which are largely transmitted by ingestion of *T. solium* eggs excreted in human faeces, should be decreasing. Given that according to the World Health Organization the period between infection and symptoms ranges between months and years, with a median of 3·5 years, it would be unlikely to expect the Swachh Bharat Abhiyan campaign to have had any impact yet; however, earlier campaigns and more general societal trends may have had an effect. However, data addressing any alteration in cysticercosis prevalence over time are lacking. The purpose of this study was thus to conduct a retrospective investigation of at any alteration in the prevalence of cysticercosis and NCC by examining records and serological data from the 17-year period from 1998 to 2014, available at the Department of Medical Parasitology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. In addition, demographic factors (age and gender) associated with cysticercosis over time were considered to be useful to investigate, particularly as they may identify risk groups and inform future control efforts.

METHODS

Study site

PGIMER is a medical and research institution located in Chandigarh, India. It has educational, medical research and training facilities for its students, and, as it is a tertiary-care referral hospital, patients come from the surrounding area, including the various adjoining states, as well as from Chandigarh city itself. Chandigarh is located in Punjab and Haryana, near the foothills of the Sivalik range of the Himalayas, in northwest India. Chandigarh had a population of over 1 million in 2011, giving a density of about 9252 persons/km^2 (up from 7900 in 2001). The 2016 Swachh Bharat Survey ranked Chandigarh as being the second cleanest city in India.

Diagnosis of cysticercosis and NCC at PGIMER

Cysticercosis and NCC are diagnosed at the Department of Medical Parasitology in PGIMER using an in-house enzyme-linked immunosorbent assay (ELISA), based on IgG capture using crude soluble extract antigen of *T. solium,* which has not been modified over the years, and is used for detection in both serum and cerebrospinal fluid. The sensitivity and specificity of this test in this laboratory have previously been published as being 78·9% and 83·3%, respectively [9].

Serum samples are sent to the parasitology laboratory on suspicion of NCC or cysticercosis according to clinicians examining patients at the various outpatient departments. The criteria for suspicion are not stipulated, but medical cards indicate headache, epilepsy, and seizures. There is no reason to believe that the criteria have changed over time. Imaging may also have been conducted. Most requisitions for serology do not provide clinical information.

Data compilation

Data from the 17-year period from 1998 until 2014 were extracted from stored paper records, and entered into an electronic database. All records were included for which there was a request for serology for cysticercosis and a result of this test, regardless of completeness of data. Due to the number of records for which data were missing, in particular clinical data, none of the entries were excluded initially, although some were excluded during analysis. Data were entered by one individual, with cross-checking of data entry by one of two other researchers.

Statistical analysis

Descriptive data were collected from the database. Considerable amounts of data (regarding age, gender, and clinical signs and symptoms) were not provided in the serology request sheets. For comparisons of distribution of seropositivity by gender and age, cases for which the relevant information was not supplied were excluded from the analyses. The numbers of cases excluded due to missing data are listed in the appropriate sections in the Results section.

Associations between seropositivity and gender and age group were investigated using contingency table analysis (Fisher's exact test). Changes in seroprevalence over time were investigated by dividing the data into two approximately equal periods of time (the first 8 years, 1998–2005 and the subsequent 9 years, 2006–2014) and investigated by contingency table analysis (Fisher's exact test). Logistic regression analysis was used to determine any alteration in likelihood of obtaining a positive result.

RESULTS

Overall seropositivity from 1998 to 2014, and distribution by gender and age

Among the serum samples from the 9650 patients analysed at the Department of Medical Parasitology, PGIMER between 1998 and 2014, 1716 patients were seropositive for cysticercosis, giving an overall seroprevalence of 17·8% in this group (patients whom the medical practitioners suspected of suffering from cysticercosis).

Of the 9650 patients, gender information was provided for 8773 patients, with 61% male (5352 patients) and 39% female (3421 patients) – no gender data were provided for 877 patients (all of whom were seronegative), and these were not included in the analysis. Of the 1716 positive cases, 945 (55·1%) were male and 771 (44·9%) were female (Table 1). However, as many more males than females were investigated by serology for cysticercosis, there is a skewed gender distribution regarding the samples analysed, and contingency table analysis using Fisher's exact test indicates a significant association with gender and seropositivity, with women significantly more likely to be seropositive than males (22·5% of samples from females analysed were positive, compared with 17·7% of samples from males analysed being positive; $P < 0·0001$).

With respect to age group, data were available for 7075 of the 9650 patients tested, with 6277 (88·7%) being children and 798 (11·3%) adults. Age data were missing for both seropositive and seronegative cases, and these cases were excluded from the

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Table 1. *Patients' samples analysed for cysticercosis, 1998–2014. Gender distribution in positive samples*

Year	Patient samples, *N*	Patient samples seropositive, *n* (%)	Male samples seropositive, *n*	Female samples seropositive, *n*
1998	1014	112 (11)	62	50
1999	923	117 (12·7)	69	48
2000	932	216 (23·2)	127	89
2001	783	195 (24·9)	107	88
2002	678	154 (22·7)	87	67
2003	461	121 (26·3)	66	55
2004	499	117 (23·5)	59	58
2005	494	139 (28·2)	78	61
2006	456	87 (19·1)	58	29
2007	508	67 (13·2)	39	28
2008	513	86 (16·8)	44	42
2009	421	52 (12·4)	22	30
2010	411	48 (11·7)	22	26
2011	426	50 (11·7)	22	28
2012	401	78 (19·5)	38	40
2013	416	55 (13·2)	34	21
2014	314	22 (7)	11	11
Total	9650	1716 (17·8)	945	771

analyses. Of the 1716 positive patients, age data were available for 1367 patients, and showed a distribution of 1172 children aged $\leqslant 17$ years (85·7%) and 195 (14·3%) adults. However, as many more children were investigated by serology for cysticercosis, there is a skewed age distribution for samples analysed and contingency table analysis using Fisher's exact test indicates a significant association with age group and seropositivity, with adults more likely to be seropositive than children (19% of children tested and age group provided were seropositive, compared to 24% of adults tested; $P < 0·0002$).

The mean age (±standard deviation) of children diagnosed with cysticercosis was $7·52 \pm 3·49$ years and for adults was $35·09 \pm 12·55$ years.

Changes in seropositivity from 1998 to 2014

The trend in the number of cases referred for serological testing for cysticercosis/NCC indicates a decline with time, with fewer patients presenting with symptoms indicative of these conditions in 2014 than 1998 (less than one third of the number in 2014 than in 1998). The overall trend in the number of patients found to be seropositive, and also the proportion of patients tested found to be seropositive, shows, on the whole a decrease over time (Fig. 1). The highest spot prevalence by year was in 2005, in which there were 2·05 cases/1000 population being investigated, and the lowest was in 2014, in which there were 0·70 cases/1000 population studied.

The division of the data into two groups (1998–2005, 2006–2014) is described in Table 2. The first period had a total of 5784 patient samples, of which 1171 were positive (20·3%). During the second period, 3866 samples were received, of which 545 were positive (14·0%). The overall decline in patient numbers from the previous to the more recent period was 1918 (67%), and the rate of decline of seropositive samples was 30·4% by comparing the seropositive percentages.

For both children and adults, logistic regression indicated that the likelihood of a positive result decreased over time (children: 95% confidence interval −1·28 to −1·15; adults: −0·91 to −0·57).

DISCUSSION

Data obtained from this retrospective analysis of 17 years' worth of cysticercosis serological data from PGIMER, Chandigarh, India, indicates an overall spot prevalence of between 2·05 and 0·07 cases/1000 population investigated, depending on year. The overall prevalence of 17·8% in suspected patients, in a country of low meat consumption and a city considered to have relatively good infrastructure and levels of cleanliness is noteworthy.

It should be noted that the sensitivity and specificity of the diagnostic tests used are 78·9% and 83·3%,

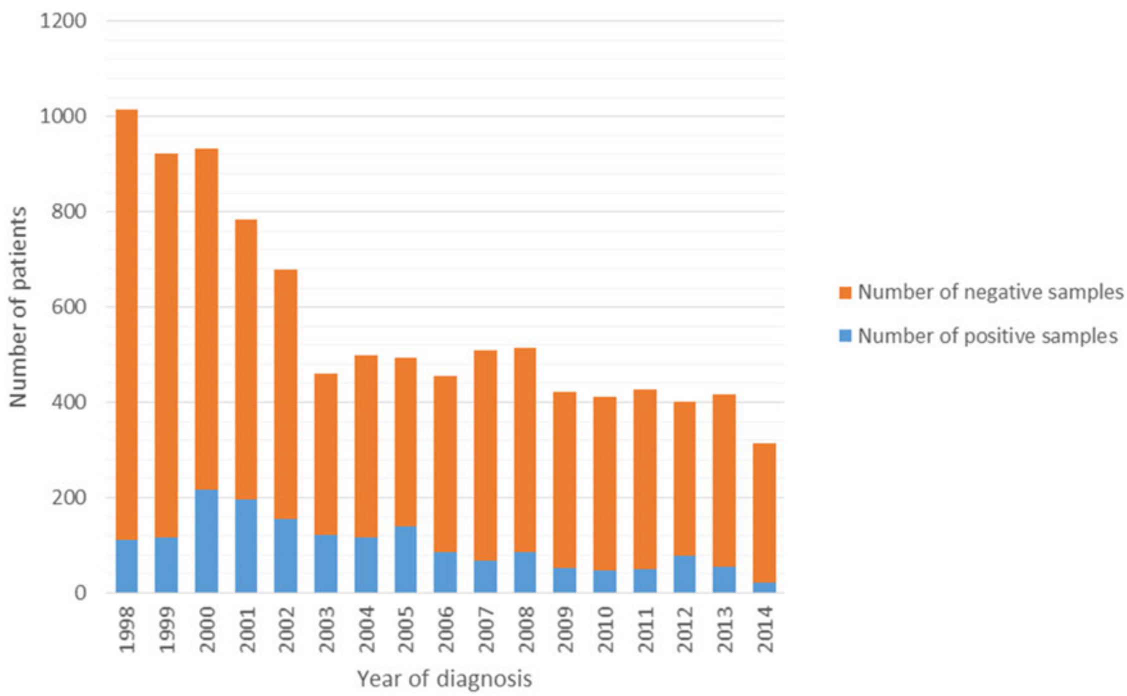

Fig. 1. Numbers of suspected cases of cysticercosis at the Postgraduate Institute of Medical Education & Research, Chandigarh, India per year from 1998 to 2014 and investigated by serology. Seropositive cases are shown in blue.

Table 2 *Patients' samples analysed for cysticercosis, divided by time into two groups (period 1: 1998–2005; period 2: 2006–2014)*

	Period 1	Period 2	Total
Positive samples	$n = 1171$ (20·3%)	$n = 545$ (14·0%)	1716
Negative samples	$n = 4613$ (79·8%)	$n = 3321$ (85·9%)	7934
Total	5784	3866	9650

respectively [9]; false positives may be detected due to cross-reactivity. Although techniques such as electro-immunotransfer blot are considered to have a higher specificity (e.g. [10]), ELISA continues to be the routine test at PGIMER, due to pragmatic reasons regarding implementation.

These data are more similar to the data previously reported from southern India [8] than from northern areas of India [6, 7]. However, both these studies concentrated solely on epilepsy populations and NCC, whereas in our study a number of clinical signs indicative of cysticercosis or NCC could have resulted in a serological test, and no distinction between NCC and cysticercosis was made. Nevertheless, given that NCC is more likely to result in symptoms that result in a serological test being requested (seizures), it could be expected that the majority of seropositive cases suffer from NCC.

The contrast between the relatively lower seropositivity described here compared to other areas of Northern India, might also indicate the relatively good infrastructure in Chandigarh and also a lower prevalence of taeniosis. Data on taeniosis prevalence in this area is lacking, probably because this infection is often asymptomatic and diagnosis would often be incidental or found during faecal screening for other parasites. However, a review from 2005 indicates a prevalence of taeniosis in India of around 2% [11]. As transmission of cysticercosis is almost exclusively dependent on environmental contamination with human faeces, infrastructure that minimizes open defecation is important for reducing transmission. In the study from Uttarakhand [7], the authors note the lack of basic infrastructure, with <11·5% of homes having an in-built toilet facility and 42% of families using soil or ash for hand washing; over 90% of families were farmers. Chandigarh, in contrast, has been described as the 'wealthiest town in India', with the highest *per capita* income in the whole of India (see http://chandigarh.gov.in/knowchd_redfinechd.htm).
In terms of sanitation, in addition to private toilets, the Chandigarh city corporation provides public

toilets (98 built public toilets and 42 mobile toilets) (see http://mcchandigarh.gov.in/healthdept.htm). This improved sanitation and infrastructure are likely to be major contributors to the relatively low burden of cysticercosis in this population, which, nevertheless, is still high compared with, for example, Western Europe, where cysticercosis cases diagnosed seem to be almost exclusively imported (not autochthonous) [12]. Nevertheless, despite the improvements in sanitation and infrastructure in Chandigarh, according to the 2011 census data, about 10% of Chandigarh's population of ~1 026 500 live in slum areas (slums are defined as 'residential areas where dwellings are unfit for human habitation because they are dilapidated, cramped, poorly ventilated, unclean, or any combination of these factors which are detrimental to the safety and health'). Although the slum-dwelling population decreased from 11·89% in 2001 to 9·7% in 2011, the overall rise in population by 17% means that even in 2011 a substantial number of people (around 971 000) were living in slums. In addition, according to the 2011 status, around 0·4% of Chandigarh's population was classified as homeless (defined as 'live on footpath or without any roof cover'). These individuals are obviously at risk for any environmentally transmitted infection. It should also be noted that PGIMER also accepts patients from areas outside the city of Chandigarh (and, as a government-supported hospital, is among the cheapest healthcare providers in the area), and some of the positive patients may well have originated from regions in which the infrastructure is not as good.

It is generally considered that the effective elimination of cysticercosis from Western Europe was due to the improvement in pig-rearing conditions and the implementation of meat inspection procedures around 1920 [13]. It is possible that exposure of pigs to infection in Chandigarh may have been reduced through the introduction of initiatives from around 2006 for improved pig husbandry, but quantitative data are not available, and projects described in the media tend to be mostly associated with breeding. Although meat inspection is mandatory in India, it is clear that in some areas it is carried out in a somewhat desultory manner (e.g. [14]) – and it is of probable relevance that a ranking exercise among a group of biomedical students in Chandigarh revealed that the profession of veterinarian is considered to be of very low status [15]. However, even if conducted carefully, routine carcass inspection is likely to miss light infections [16]. Thus, although meat consumption in India in general, including Chandigarh, is very low (over 90% of the population of Chandigarh are either Hindu or Sikh, for which religions vegetarianism is not explicitly compulsory, but nevertheless implied), there is, however, clearly sufficient to enable the *T. solium* life-cycle to be maintained. About 8000 Christians are resident in Chandigarh, However, the extent to which pork is consumed in this small group is not known. Furthermore, a study in Goa, Southwest India was unable to find an association between seropositivity for cysticercosis and pork consumption or religion [17].

Studies on the extent of pig cysticercosis in India are very few; one study from urban slums in Punjab state (in which Chandigarh is located), found that out of 519 pigs from small slaughter shops (shops that sell meat from animals that are slaughtered on the premises as the customer waits), 22 (4%) contained cysticerci of *T. solium* [18]. It should also be noted that pig meat tends to be consumed by those of lower socioeconomic status in India – who are also those people with limited access and knowledge regarding proper sanitation. Nevertheless, given the distribution of slum areas in Chandigarh and the widespread shedding of *T. solium* eggs, no specific stratum of society can be considered as completely unexposed.

The age associations with cysticercosis cases identified in this study reflect the biological and clinical situation. Although from a numbers point of view, more cases were identified in children, the considerably higher number of samples from children examined (and, indeed, attending the various outpatient departments at PGIMER) disguises the fact that adults were more likely to be diagnosed with cysticercosis among those presenting with symptoms. As symptoms of cysticercosis usually develop after some time (months to years) post-infection, and may not immediately be diagnosed or treatment sought, it is to be expected that adults presenting with symptoms indicating a need for serological testing are more likely to be seropositive than children. Studies from elsewhere have also noted that increased age was a significant risk factor for cysticercosis (e.g. [19–22]). Furthermore, most cases of NCC in children are seen after age 5 years (although some cases do occur in pre-schoolers) and occasionally infants [23], and our results from children bear this out.

The gender association is interesting. Again, from a numbers perspective, more males were infected than females, but as more samples from males were analysed, this hides the fact that of the males and females with symptoms suggesting cysticercosis or NCC, females were more likely to be seropositive. The data suggest that women are less likely than men to seek medical assistance, perhaps due to a variety of social factors, and this may bias the data yet further. Indeed, a study from Chandigarh concerned with healthcare-seeking behaviour of tuberculosis patients indicated that women are less likely than men to seek timely medical care, generally due to financial constraints and initially using home remedies or medicines [24].

Studies from other areas of the world, e.g. Kenya [25], Tanzania [22], and Bolivia [19], also suggest that cysticercosis is associated with female gender, but studies from other countries find the opposite association, with males more likely to be seropositive for cysticercosis, e.g. in Burkina Faso [21]. Studies from other countries find no gender association, e.g. in Zambia [20]. Presumably, these differences reflect cultural variations; for example, the female bias in Kenya was suggested to be associated with women being more likely to be exposed to faecal contamination in the environment as they provide most of the agricultural labour in smallholder farming in Kenya [25]. The male bias noted from the Burkina Faso study suggests that poor hand hygiene, consumption of inadequately cleaned fresh produce, or eating meals outside the home potentially prepared by food-handlers with taeniasis may be relevant [21]. The reasons for the gender difference identified in our study are unclear. Although the female share of the labour force in agriculture in India is around 30%, calculated as the total number of women economically active in agriculture in India divided by the total population economically active in agriculture [26], at the same time over 75% of Indian women are engaged in working in the fields, particularly low-end jobs like hand-sowing, transplanting, and weeding [27] that may be more like to result in exposure to faecal contamination. In addition, preparation of meals tends to be largely associated with women in a household in India, and thus the potential for handling contaminated foodstuffs and water is also greater. Another potential source of exposure is through cleaning tasks, particularly dealing with faecal accidents from family members, especially children, and sweeping in general. The jharu broom, commonly used by women in India, means close contact with contaminated areas as it is generally used in a crouching position. The Organisation for Economic Co-operation and Development (OECD) Gender Data portal reported in 2014 that on a global basis, Indian men do the least household work in the world (http://www.oecd.org/gender/), and may therefore be less likely to have this exposure route. Interestingly, however, a recent study demonstrated that men dominate in working with municipal solid waste in Chandigarh, which may also result in greater exposure to *Taenia* eggs, as well as other health hazards [28].

The apparent reduction in cysticercosis over time is of particular interest. The slight increase in percentage of samples seropositive during 1998–2000 may simply reflect lack of experience with the newly introduced assay, although this cannot be proven. Thereafter the numbers and proportion remained approximately steady, peaking proportion-wise in 2005, and thereafter falling slowly, both proportion-wise and number-wise, with the lowest (for both) in 2014. The number of samples analysed also fell steadily over the whole period, indicating that fewer patients are consulting clinicians with symptoms indicative of cysticercosis.

The reasons for these declines can only be speculative, but likely represent improvements in infrastructure such as sewage and sanitation, and slaughter routines. Although there have been concerted campaigns to clean India, given the prolonged period between infection and diagnosis for cysticercosis (ranging from months to years) it would seem doubtful that the more recent cleanliness initiatives would have yet made a noticeable impact on cysticercosis cases. A reduction in cases of taeniosis would reduce environmental contamination and transmission risk. This could be brought about by a focus on diagnosis and treatment of taeniosis, or by less consumption of inadequately cooked pork. However, there are no data that support these speculative possibilities. Although animal census data are compiled on a regular and regional basis in India, these provide no clues regarding possible alterations in pig husbandry in Chandigarh and surrounding areas over this period as different datasets provide contrasting information, with numbers of pigs registered in the livestock census data from Chandigarh ranging from 0 to 150 to several thousand.

As noted by Devleesschauwer *et al.* [12], in attempting to control cysticercosis, then identification of tapeworm carriers that are the source of the contamination are crucial from both an epidemiological and public health point of view. Thus, attempting to control cysticercosis is truly a One Health effort, involving medical efforts (diagnosis and treatment of *T. solium* tapeworm carriers in addition to those suffering from cysticercosis), veterinary efforts directed towards meat inspection and prevention of infection of pigs (minimizing their access to human waste), environmental health and sanitation engineers (to minimize environmental contamination with human waste), and relevant government agencies involved in implementation and evaluation of appropriate control initiatives. The importance of the One Health concept in control of *T. solium* has been mentioned in several recent studies (e.g. [29–31]), and indicates that even this combined approach is difficult due to major challenges as soon as specific control programmes end.

The apparent reduction in cysticercosis over the period of this study is encouraging, but further efforts are needed to reduce it yet further, particularly in women. Although there are no comprehensive programmes targeting NCC in India, the Swachh Bharat Abhiyan (Clean India Mission), initiated in 2014 by the current government, could provide a useful approach for prevention and control of this zoonosis in the population of India.

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DECLARATION OF INTEREST

None.

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