NOTE *Parasitology*

Age-Specificity of *Toxoplasma gondii* Seroprevalence in Sheep, Goats and Cattle on Subsistence Farms in Bangladesh

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ABSTRACT. *Toxoplasma gondii* is a zoonotic protozoan parasite that infects humans and domestic animals. In this study, the seroprevalence of *T. gondii* antibodies was investigated using serum samples collected from 83 sheep, 146 goats and 37 cattle from a dozen subsistence farms in Bangladesh. Fifty-eight out of 83 sheep (69.9%), 89 out of 146 goats (61.0%) and 10 out of 37 cattle (27.0%) were seropositive for the parasite. Seroprevalence in young goats (<1 year old) was significantly lower than that of the adult goats (>1 year old). In contrast, seroprevalence for young and adult sheep was similar. These results indicate that acquired infection with *T. gondii* occurs in this region of Bangladesh, at least among goats.

KEY WORDS: Bangladesh, goat, seroprevalence, sheep, *Toxoplasma gondii*

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Toxoplasma gondii, a zoonotic protozoan parasite, causes widespread infection in humans and domestic animals. We recently reported that domestic animals, such as cattle, sheep and goats, which are used as meat sources in Bangladesh, showed a high seroprevalence of *T. gondii* [6]. This high prevalence has not changed from that reported in 1997 [5]. Addressing this situation is important in Bangladesh, not only for effective rearing of domestic animals, but also for public health. The definitive host of *T. gondii* is the cat, and the main routes of infection in ruminant animals are ingestion of the oocysts shed by cats and trans-placental vertical transmission [1]. For effective control of *T. gondii* on subsistence farms in Bangladesh, it is necessary to determine the routes of infection among the domestic ruminants used as meat sources. However, obtaining sufficient numbers of serum samples to investigate this is relatively difficult in rural areas of Bangladesh where intensive stock farming is not common, and only a few domestic sheep, goats and/or cattle are kept by subsistence farmers using traditional non-intensive systems for animal rearing.

To address this difficulty, in this study, we made use of the 'Free Vaccination Program for Deprived Small-hold Farmers' from the Rajshahi University of Bangladesh, in which small-hold farmers are offered free vaccination of their livestock. Using this opportunity, we collected serum samples from 83 sheep, 146 goats and 37 cattle from a dozen domestic farms in a rural area, a suburb of Rajshahi city, Bangladesh (Fig. 1). During sample collection, we asked the farmers the ages of their animals. Sampling of the animals was approved by the Animal Research Committee of Gifu University and was carried out with the owners' consent. *T. gondii* antibody detection was done using a commercially available diagnostic kit for humans, Toxotest-MT (Eiken Kagaku, Tokyo, Japan). In this system, anti-*Toxoplasma* antibodies were detected via agglutination of latex particles. Although this diagnostic kit has been widely used for fundamental research in the veterinary field [3, 4, 6, 7], it has not been approved for use in animals by the manufacturer, and the cut-off values for positivity in animals have not been determined because the test was designed for toxoplasmosis diagnosis in humans (where the cut-off value is a titer of 1:32). To assess whether the same cut-off value for determining positivity is also acceptable for sheep, goats and cattle, we determined the distribution of antibody titers measured by the diagnostic kit. As shown in Fig. 2A, all 3 species (sheep, goats and cattle) showed a bimodal distribution of antibody titer across a titer of 1:16. Therefore, a cut-off titer of 1:32 or greater was considered positive for animals in this study. It has also been reported that live *T. gondii* parasites could be isolated from the muscle samples of more than 70% of goats, which were seropositive using the same kit as was used herein, but not from the seronegative animals [2]. These results strongly suggest that the criteria used to determine seropositivity are suitable not only for humans, but also for the domestic animals we investigated. Using these criteria, 58/83 sheep (69.9%), 89/146 goats (61.0%) and 10/37 cattle (27.0%) were seropositive. When differences in seroprevalence between the species were analyzed by Fisher's exact test, statistical differences were found between cattle vs.

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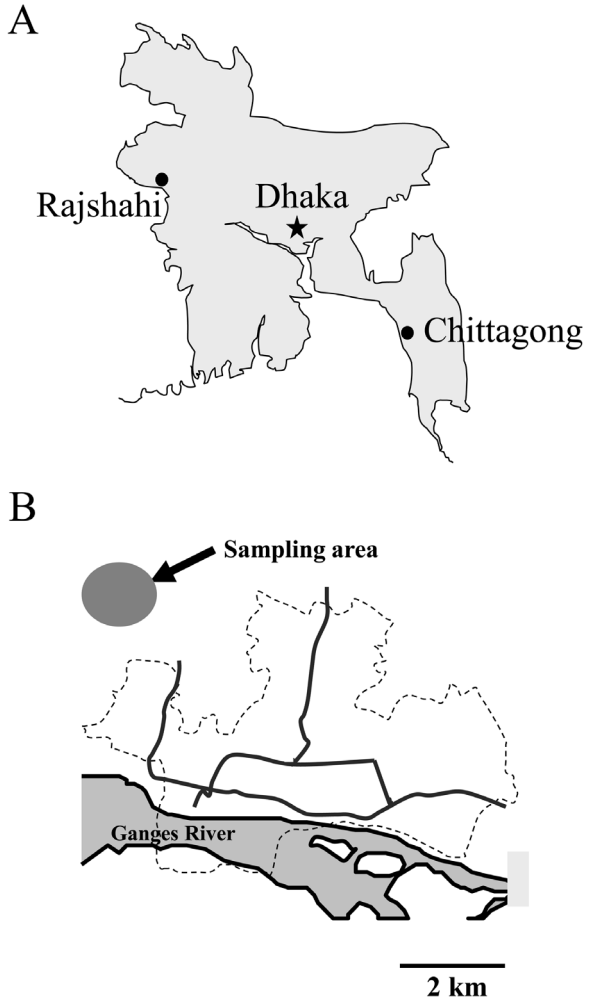

Fig. 1. Location of sampling area. (A) Map of Bangladesh. (B) Map of Rajshahi city. City limits are indicated by dot-line. Heavy lines indicate arterial streets.

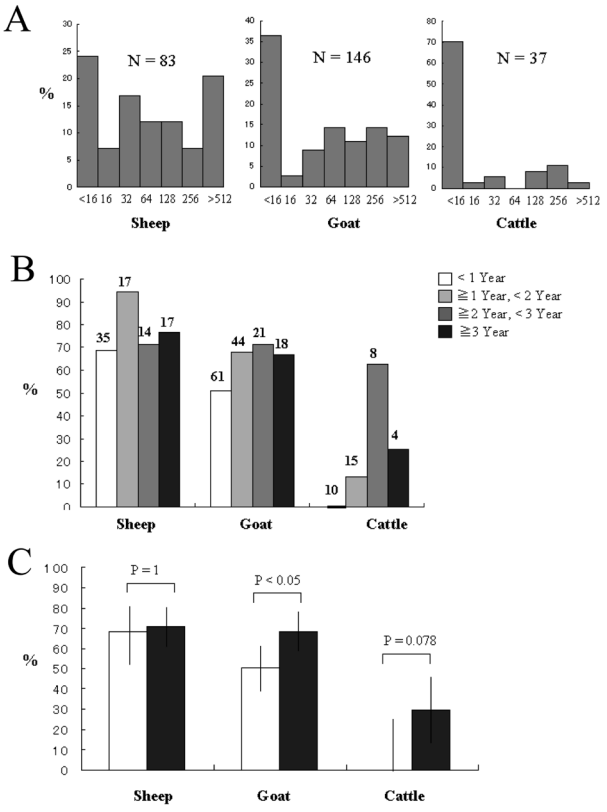

Fig. 2. (A) Distribution of antibody titers determined by the Toxotest-MT test in serum samples from 83 sheep, 146 goats and 37 cattle. Percentage in vertical axis indicates frequency of individuals showing each titer. (B and C) Age-specific seroprevalence of *T. gondii* among the 83 sheep, 144 goats and 37 cattle used in this study. Inserted numeric characters indicate the total number of animals in each age-bracket. Two goats whose ages were not known were omitted from the study. (B) Seroprevalence according to age-range. Percentage in vertical axis indicates seroprevalence in each age-group. (C) Comparison of seroprevalence between young animals (open bars) and adults (shadowed bars). Seroprevalence in each age-group and the 95% confidence interval of seroprevalence calculated using F-distribution were shown. Percentage in vertical axis indicates seroprevalence in each age-group.

goats ($P<0.01$) and cattle vs. sheep ($P<0.01$). We have previously reported a similar trend in another Bangladeshi city [6]. Although the difference of susceptibilities to *T. gondii* between ruminant species has not yet known well, sheep and goats might be more susceptible to *T. gondii* infection than cattle. There is also a possibility that the higher seropositivity seen in sheep and goats is influenced by the fact that they tend to roam more freely in the streets than cattle and thus have greater access to domestic cat feces.

Next, we calculated the seroprevalence of *T. gondii* in sheep, goats and cattle according to their age. As shown in Fig. 2B, seroprevalence in goats and cattle less than 1-year-old appeared to be lower than that of the older animals. In contrast, no clear age-specificity was observed in sheep. To examine this more objectively, differences in the young (<1 year old) and adult (≥1 year old) animals were analyzed by Fisher's exact test. We found that seroprevalence in the young goats was statistically lower than that in the adult goats (Fig. 2C). We found no statistically significant difference between seropositivity in the young and adult cattle ($P=0.078$), but this may be caused by the small sample size. There was no significant difference in seropositivity between the young and adult sheep. Considering that the oldest goat we examined was just 5-year-old, it is most likely that acquired infection occurs rapidly over only a few years in the goats. In the United Kingdom, trans-placental vertical infection is thought to be the main route of *T. gondii* infection among ruminants with acquired infection, with oocyst transmission playing a relatively minor role [1, 8]. However, our observations indicate that acquired infection rates are not negligible in the area of Bangladesh we studied. Prevention of acquired infection is considered essential for eradication

or control of *T. gondii* among animals used for beef and goat meat in Bangladesh. For this purpose, instigation of control measures for environmental pollution by oocysts and development of a better organized system for rearing animals would benefit this area. However, our results also indicate that seroprevalence in sheep has already reached its saturation point at the age of 1 year (Fig. 2B and 2C). This implies that transplacental infection may play a major role in *T. gondii* infection in sheep, although oocyst ingestion cannot be ruled out. Free-ranging sheep owned by domestic-scale farmers often cluster together to form a flock, so it is possible that nearly all sheep in this area of Bangladesh have ingested oocysts during their first year of life. A prospective cohort study to determine the timing of seroconversion is necessary to evaluate the relative importance of trans-placental and acquired infection among sheep in this area.

In rural areas of developing countries, it is common for each small-hold farmer to keep only a few domestic animals. However, domestic animals, such as these, have rarely been studied, because of the difficulty with systematic collection of specimens. To our knowledge, this study is the first to report on age-specific seroprevalence of *T. gondii* among ruminants owned by subsistence farmers in Bangladesh. Our observations provide evidence for developing countries that it is hoped to initiate improvements in hygiene related to animal husbandry and meat production on rural subsistence-level farms.

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