Evidence of *Toxoplasma gondii* Exposure among Horses in Korea

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**ABSTRACT.** The present study investigated the seroprevalence of *Toxoplasma gondii* (*T. gondii*) antibodies by ELISA in horses reared in Korea. Serum samples were collected from 2009 through 2013 from 816 horses reared in Korea. Analysis was performed using a commercial toxoplasmosis ELISA kit to detect anti-*T. gondii* antibodies. Overall, 24 out of 816 horses (2.9%) were seropositive for *T. gondii*. The result was analyzed by age, gender, breed and region. Significant differences were observed according to breed and region (P<0.05). This is the first nationwide serological investigation of *T. gondii* in horses reared in Korea. The study results reveal that *T. gondii* occurs nationwide in Korean horses.

**KEY WORDS:** ELISA, equine, Korea, prevalence, toxoplasmosis

NOTE: *Parasitology*

*Parasitology* was interpreted as follows: negative (<40%), doubtful (40–50%) and positive (>50%).

Thus, the purpose of the present study was to investigate the seroprevalence of *T. gondii* on a nationwide scale in horses raised in ROK.

Blood samples were collected from 2009 through 2013 from the jugular vein of 816 horses reared in Korea. The use of experimental animals was approved by the Kyungpook National University Institutional Animal Care and Use Committee (Approval #KNU-200900501). The breeds of horses included the Thoroughbred (69.5%, 567/816), Korean native pony (13.4%, 109/816), warmblood (7.5%, 61/816) and mixed breed (9.7%, 79/816). The study area included all parts of the country and horse farms in each province and was divided into four regions (northern, central, southern and Jeju-do) (Fig. 1). Data regarding age, gender, breed and region of origin of each animal were collected; data were classified as “unknown” in cases of insufficient data. Serum was separated by centrifugation and stored at −20°C until use.

The anti-*T. gondii* antibodies in each serum sample were detected using a commercial toxoplasmosis multi-species ELISA kit (IDvet, Montpellier, France). All experimental steps were conducted according to the manufacturer’s instructions. Specificity and sensitivity of the multi-species kit are 99.9% and 84%, respectively, in the pig [1]. To determine seroprevalence, optical density (OD) values were measured at 450 nm, and the positive percentage of the sample (S/P) was calculated according to the manufacturer’s instructions as follows: $S/P = 100 \times \frac{(OD_{\text{sample}} - OD_{\text{negative control}})}{(OD_{\text{positive control}} - OD_{\text{negative control}})}$. S/P was interpreted as follows: negative (<40%), doubtful (40–50%) and positive (>50%). In this study, doubtful results were considered negative.

For statistical analysis, chi-square and Fisher’s exact tests were used. The data of the “unknown” group were disregarded in the chi-square and Fisher’s exact tests. Statistical
analyses were carried out using SPSS 21.0 (IBM Corporation, Armonk, NY, U.S.A.), and \( P \)-values of <0.05 were regarded as significant. A 95% confidence interval for all estimates was calculated.

Among the 816 tested horses, 24 (2.9%) were found to be seropositive for anti-\textit{Toxoplasma gondii} antibodies by ELISA (Table 1). With regards to age, the seroprevalence was 1.9% (5/269), 3.6% (9/251), 1.0% (2/202) and 8.5% (8/94) for horses aged <5 years, 5–10 years, >10 years and of unknown age, respectively. Regarding gender, 1.3% (2/160), 2.8% (8/282), 2.1% (6/280) and 8.5% (8/94) of the samples were seropositive in the male, female, castrated and unknown groups, respectively. There was no significant difference among the groups in age and gender.

Regarding breed, 1.9% (11/567), 7.3% (8/109), 4.9% (3/61) and 2.5% (2/79) of the samples were seropositive in the Thoroughbred, Korean native pony, warmblood and mixed breed, respectively. According to region, 2.7% (8/301), 2.2% (4/178), 1.6% (4/243) and 8.5% (8/94) of the samples were seropositive in the northern, central, southern and Jeju-do regions, respectively. There were significant differences according to breed and region (\( P < 0.05 \)). In this study, breed of horse was associated with region of collection; most of the samples (94/109) from Korean native ponies were collected in Jeju-do, and the highest seroprevalence (8.5%, 8/94) occurred in Jeju-do in regional analysis. In addition, all of the Korean native pony samples that were seropositive were collected in Jeju-do (data not shown). Although additional investigation is needed that takes into account environmental conditions, genetic factors and food preferences of the residents, an association may exist between seroprevalence and region, particularly with respect to Jeju-do, for \textit{T. gondii} infection.

The higher prevalence in Jeju-do may be explained by two factors. First, horses in Jeju-do are allowed to graze freely, whereas mainland horses are reared semi-intensively, i.e., horses are reared in a confined system with limited grazing time. Thus, horses in Jeju-do are likely to more easily acquire parasites from the natural environment. Second, the infection status of the definitive host, i.e., cats, in Jeju-do appears higher than that on the mainland. Although the studies were not conducted in the same year, the seroprevalence of \textit{T. gondii} among cats in Jeju-do was 37.0% despite a very small population [6]; this is in contrast to 15.8% (69/456) in Seoul [8], 16.1% in Gyeonggi-do [4] and 13.1% in Jinju [10], which are located on the mainland. Thus, the higher prevalence in horses could be related to the higher prevalence in stray cats. However, a further epidemiological comparative study between horses and cats and investigation of distribution according to that of \textit{T. gondii} in the natural environment are needed.

This is the first nationwide large-scale serological study on the prevalence of \textit{T. gondii} in horses raised in ROK. The overall positive rate of \textit{T. gondii} antibodies in horses is low, and there is higher prevalence of \textit{T. gondii} among horses in Jeju-do than mainland regions. The high prevalence in Jeju-do is significant, because most of the production and consumption of horse meat in ROK occur in Jeju-do [7].

\begin{table}
\centering
\caption{Seroprevalence of \textit{Toxoplasma gondii} in 816 horses according to age, gender, breed and region}
\label{table1}
\begin{tabular}{|l|l|l|}
\hline
Group & No. positive / total (%) & 95\% CI\textsuperscript{a}) \\
\hline
\hline
\textbf{Age} & & \\
<5 y & 5/269 (1.9) & 0.2–3.5 \\
5–10 y & 9/251 (3.6) & 1.3–5.9 \\
>10 y & 2/202 (1.0) & 0–2.4 \\
Unknown & 8/94 (8.5) & 2.9–14.1 \\
\hline
\textbf{Gender} & & \\
Male & 2/160 (1.3) & 0–3.0 \\
Female & 8/282 (2.8) & 0.9–4.8 \\
Castrated & 6/280 (2.1) & 0.5–3.8 \\
Unknown & 8/94 (8.5) & 2.9–14.2 \\
\hline
\textbf{Breed\textsuperscript{b})} & & \\
Thoroughbred & 11/567 (1.9) & 0.8–3.1 \\
Korean native pony & 8/109 (7.3) & 2.4–12.2 \\
Warmblood & 3/61 (4.9) & 0–10.3 \\
Mixed & 2/79 (2.5) & 0–6.0 \\
\hline
\textbf{Region\textsuperscript{b})} & & \\
Northern & 8/301 (2.7) & 0.8–4.5 \\
Central & 4/178 (2.2) & 0.1–4.4 \\
Southern & 4/243 (1.6) & 0.1–3.3 \\
Jeju-do & 8/94 (8.5) & 2.9–14.2 \\
\hline
\textbf{Total} & 24/816 (2.9) & 1.8–4.1 \\
\hline
\end{tabular}
\textsuperscript{a}) CI, confidence interval, b) \( P < 0.05 \).
\end{table}
Our findings provide an update on the status of *T. gondii*, particularly in the horse and may serve as the basis of future investigations on the significance of this parasite in ROK. In addition, continuous monitoring and implementation of integrated control strategies of *T. gondii* infections are needed to prevent human infections and public health considerations.

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