Short Communication

The seroprevalence of Japanese encephalitis virus in goats raised in Korea

Dong-Kun Yang^{1,*}, Chang-Hee Kweon¹, Byoung-Han Kim¹, In-Jin Hwang¹, Mun-Il Kang¹, Byung-Jae So¹, Kyoung-Oh Cho²

¹National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-824, Korea

Japanese encephalitis virus (JEV) causes a mosquitoborne viral zoonosis that is becoming increasingly important to public health in east and south Asia. Although JEV is primarily associated with reproductive failure in swine, JEV infection can cause fever and headache in humans and is associated with aseptic meningitis and encephalitis. The exact mode of transmission, including host range and possible source of viral amplification within livestock, is still not completely clear. This study consisted of a serological survey of JEV infection in goats. A total of 804 goat serum samples were collected from 144 farms in Korea between May 2005 and May 2006. The incidence of positive cases was 12.1% (97 out of 804 goats). The seroprevalence of JEV infection in the 144 farms screened was 31.3% (45/144), indicating that JEV infection is frequent in goat farms in Korea. In addition, three districts of Korea (mainly in the southern region) had a higher seroprevalence of JEV compared to other areas. The results suggest that goats could be monitored epidemiologically as a sentinel animal for JEV transmission in Korea.

Key words: goat, JEV, seroprevalence

Japanese encephalitis (JE) is a mosquito-borne viral zoonosis that is becoming increasingly important in terms of public health. Japanese encephalitis virus (JEV), a member of the genus *Flavivirus* in *Flaviviridae*, is an emerging virus that is spreading to new areas. Several species of mosquito, including *Culex tritaeniorhynchus* in Asia, are thought to be vectors for JEV. Domestic and wild animals, including pigs, horses, cattle, sheep, goats, pigeons, chickens, gray herons, and reptiles, are susceptible to the virus. Adult swine, horses, cattle, and sheep usually do not manifest clinical symptoms of the disease, but they may serve as viral amplifiers [3]. Seroepidemiological surveys of JEV in pig

In this study, we investigated the seroprevalence of JEV in domestic goats to determine a more exact JE infection rate and improve our understanding of its transmission in the period from October to March. The seroprevalence survey consisted of a total of 804 goat serum samples from seven provinces from 144 farms in Korea between May 2005 and May 2006 [Gyeonggi (n = 59), Gangwon (n = 31), Chungbuk (n = 103), Jeonnam (n = 186), Jeonbuk (n = 224), Gyeongbuk (n = 91), and Gyeongnam (n = 110)]. Most of the samples were collected between October 2005 and March 2006. In order to estimate the JEV antibody status of the goat sera, the HI test was performed in 96-well microtiter plates using the standard method [2]. Viral antigens were prepared from suckling mice brains infected with the Nakayama strain using the sucrose-acetone extraction method [2]. Briefly, the infected suckling-mouse brain was homogenized with 5 volumes of 8.5% sucrose solution and the homogenate was added to 20 volumes of chilled acetone. After shaking vigorously, the milky supernatant was discarded and an equal volume of acetone was added to the bottle. This preparation was incubated for 1 h at 4°C to dehydrate the sediment. The supernatant was discarded and the sediment was dried using a vacuum pump. The dried antigen was suspended in saline. After centrifugation for 10 min at 10,000 rpm, the supernatant was used as the HI test antigen. The serum specimen was pretreated with kaolin to remove any non-specific inhibitors and then adsorbed with washed goose red blood cells to eliminate natural agglutinin. An HI

populations have been conducted as part of the preventative

measures against JE in several countries, including Korea

[1,9-11]. Since the hemagglutination inhibition (HI)

antibody to JEV infection in pigs is long lasting, and pigs

serve as viral-amplifying hosts, serological sampling of pigs

may not show the exact prevalence of JE in a given period.

The incidence of JEV-positive cases was 12.1% (97 out of 804 sera), and 45 of the 144 farms tested had positive cases. The regional distribution of positive JEV cases was 20.0 (22/110), 16.1 (30/186), 15.6 (35/224), 5.8 (6/103), 5.1 (3/59), 1.1% (1/91), and 0% (0/31) in Gyeongnam, Jeonnam,

titer of 1:20 or greater was considered positive.

²Veterianry Medical Research Center, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea

Table 1. Regional distribution of JEV antibodies from goats in Korea

| Designation | Province | | | | | | | | |
|-------------|----------|---------|-------------|-------------|---------------|---------------|--------------|--------|--|
| | Gyeonggi | Gangwon | Jeon buk | Jeon nam | Gyeong buk | Gyeong nam | Chung buk | Total | |
| Farm | 3/12 | 0/1 | 11/35 | 15/27 | 1/20 | 11/24 | 4/25 | 45/144 | |
| (%) | 25.0 | 0 | 31.4 | 55.6 | 5.0 | 45.8 | 16.0 | 31.3 | |
| Individual | 3/59 | 0/31 | 35/224 | 30/186 | 1/91 | 22/110 | 6/103 | 97/804 | |
| (%) | 5.1 | 0 | 15.6 | 16.13 | 1.1 | 20.0 | 5.83 | 12.1 | |

Table 2. Distribution of HI antibody titers among positive herds

| Designation - | HI antibody titer | | | | | | | | | |
|---------------|-------------------|--------|--------|-------|-------|-------|-------|--|--|--|
| | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | Total | | | |
| No. positive | 37 | 32 | 16 | 8 | 4 | 0 | 97 | | | |
| (%) | (38.1) | (33.0) | (16.5) | (8.3) | (4.1) | (0) | (100) | | | |

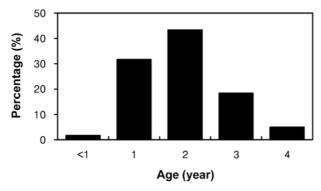


Fig. 1. Distribution of HI titers by age among goats that were seropositive against JEV.

Jeonbuk, Chungbuk, Gyeonggi, Gyeongbuk, and Gangwon province, respectively (Table 1). In addition, while there were no positive reactions from British Saanen goats imported from Australia and raised in Gangwon province (0/31), the incidence in Korean native black goats was 12.5% (97/773), with titers ranging from 1:20 to 1:320(Table 2). The highest prevalence of seropositive animals was observed in 2-year-old goats, of which 60 out of 97 had positive sera (Fig. 1). Since the HI antibody titer to JEV is not persistent in goats and lasts only about 4 weeks, it is thought that the age of the goats is not important when determining the antibody positive rate and titer, and that only the abundance and distribution of the mosquito vector are thought to be important for the JEV infection of goats during the experimental period. Of the 520,000 goats currently being raised in Korea, most are Korean native black goats that are used for meat production, as well as some British Saanen that are used for milking. Although several diseases associated with goats, such as rotavirus and bovine viral diarrhea infections [4,5] have been reported, no nationwide seroepidemiological survey of arboviral infection in goats has been reported. A serological survey of JEV

infection in domestic animals, including sheep and goats, was carried out in 1956 in Korea, but the ovine serum samples were collected only in Kyongju city and the positive rate for JEV was 21.7% (26/120) in goats [6]. In this study, three districts of Korea, primarily in southern regions, showed a relatively high seroprevalence of JEV. The results suggest that JEV is actively transmitted in the southern regions of Korea from October to March (winter). Epidemiologically, goats older than one year were more likely to be exposed to JEV than younger goats. Rajendran et al. [8] reported that HI titers against JEV in goats were low, and these levels declined to undetectable levels by approximately 4 weeks after seroconversion. In addition, Peiris et al. [7] reported that the JE seroprevalence in cattle and goats was a better predictor of the human infection risk than the porcine seroprevalence. Therefore, we believe that goats would serve as a good sentinel animal for serological monitoring of JEV infection in domestic animals because they are currently not vaccinated against JEV in Korea. A sero-monitoring system for goats and swine should provide a clear picture of the epidemiological characteristics of JEV transmission in the natural environment. Further work is required to determine which species of mosquito serve as vectors of JEV transmission in goats.

Acknowledgments

The authors would like to thank Dr. Jee-Yong Park for careful reading of the manuscript and Dr. Chung-San Lee for sampling of blood. This work was supported financially by a grant from the Agricultural R&D Promotion Center (ARPC), Korea.

References

1. **Chang KJ.** Seasonal prevalence of anti-Japanese encephalitis virus antibody in pigs in different regions of

- Taiwan. J Microbiol Immunol Infect 2002, 35, 12-16.
- 2. **Clarke DH, Casals J.** Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am J Trop Med Hyg 1958, **7**, 561-573.
- 3. Fenner FJ, Gibbs EPJ, Murphy FA, Rott R, Studder MJ, White DO. Veterinary Virology. 2nd ed. pp. 447-448, Academic Press, New York, 1993.
- 4. Kim IJ, Hyun BH, Shin JH, Lee KK, Lee KW, Cho KO, Kang MI. Identification of bovine viral diarrhea virus type 2 in Korean native goat (Capra hircus). Virus Res 2006, 121, 103-106.
- 5. Lee JB, Youn SJ, Nakagomi T, Park SY, Kim TJ, Song CS, Jang HK, Kim BS, Nakagomi O. Isolation, serologic and molecular characterization of the first G3 caprine rotavirus. Arch Virol 2003, 148, 643-657.
- Lee NS, Mun JB, Kim YH, Song KC. Studies on Japanese encephalitis. VI. Survey of incidence of the antibodies against Japanese encephalitis virus among domestic animals. Res Rep Natl Inst Vet Res 1956, 4, 21-38.
- 7. Peiris JS, Amerasinghe FP, Arunagiri CK, Perera LP, Karunaratne SH, Ratnayake CB, Kulatilaka TA, Abeysinghe MR. Japanese encephalitis in Sri Lanka:

- comparison of vector and virus ecology in different agroclimatic areas. Trans R Soc Trop Med Hyg 1993, **87**, 541-548
- 8. Rajendran R, Thenmozhi V, Tewari SC, Balasubramanian A, Ayanar K, Manavalan R, Gajanana A, Kabilan L, Thakare JP, Satyanarayana K. Longitudinal studies in South Indian villages on Japanese encephalitis virus infection in mosquitoes and seroconversion in goats. Trop Med Int Health 2003, 8, 174-181.
- Ting SH, Tan HC, Wong WK, Ng ML, Chan SH, Ooi EE. Seroepidemiology of neutralizing antibodies to Japanese encephalitis virus in Singapore: continued transmission despite abolishment of pig farming? Acta Trop 2004, 92, 187-191.
- Yang DK, Kim BH, Kweon CH, Kwon JH, Lim SI, Han HR. Biophysical characterization of Japanese encephalitis virus (KV1899) isolated from pigs in Korea. J Vet Sci 2004, 5, 125-30.
- 11. Yang DK, Kim BH, Lim SI, Kwon JH, Lee KW, Choi CU, Kweon CH. Development and evaluation of indirect ELISA for the detection of antibodies against Japanese encephalitis virus in swine. J Vet Sci 2006, 7, 271-275.