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Surveillance for Equine Infectious Anemia in Jeju Island

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Abstract : Equine infectious anemia (EIA) is a worldwide infectious disease of horses and other equids. The large serological survey of EIA was performed in Jeju from 2005 through 2011. Using the conventional enzyme-linked immunosorbent assay (cELISA), a total of 10,040 animals (1,329 Jeju Ponies, 8,324 Jeju Pony-Crossbreds and 387 Thoroughbred horses) was tested at the Equine Hospital of Jeju Race Park or Jeju Stud Farm, Korea Racing Authority. This survey found no serological evidence of EIA presence in Jeju. There had been no horse and pony with antibody against EIA since 1985 and no official report on outbreak the disease. Therefore, surveillance conducted found no evidence of EIA activity in Jeju.

Key words **:** equine infectious anemia, horses, incidence, Jeju, ponies.

Equine infectious anemia (EIA) is a worldwide infectious disease of the equidae characterized by recurrent episodes of fever, lethargy, thrombocytopenia, and anemia (4,10). EIA virus (EIAV) is a macrophage-tropic lentivirus that causes a lifelong persistent infection in horses (6). Horses infected with EIAV generally experience a clinically variable disease course that is demarcated by acute, chronic, and inapparent stages of infection.

As for other lentiviral infections, an adaptive immune response is critical both in controlling acute EIAV infection and in maintaining the inapparent stage (14). Importantly, EIAV-infected horses are able to mount broadly reactive neutralizing-antibody responses that reduce levels of replicating virus during long-term inapparent infection (12).

The most important insect vectors for natural transmission are horseflies and deerflies. Definitive diagnosis is made with serologic test (10). No effective treatment or vaccine is currently available. Furthermore, infected horses remain infected for life, often without showing any recognizable clinical signs (9).

Jeju island is very important area because of the largest product of foals and Korean native ponies in Korea. The purpose of this study was to prove EIA free on Jeju Ponies, Jeju Pony-Crossbreds and Thoroughbred horses in Jeju using cELISA test.

The serological samples were collected from 1,329 Jeju Ponies (Korean native pony) and 8,324 Jeju Pony-Crossbreds and 387 Thoroughbred horses in the quarantine process to enter Jeju Race Park and Jeju Stud Farm during a 7-year period from 2005 until 2011.

The process carried out every Wednesday at the park or when the horses would enter to the farm. In Jeju Ponies and Jeju Pony-Crossbreds, the sera obtained quarantine area. Blood collections or the tests were carried out by veterinarians or technician at the Equine Hospital of Jeju Race Park. In horses, the samples were brought by local veterinarians to the Equine Hospital of the Jeju Stud Farm. The tests were carried out by veterinarians or technicians.

Conventional enzyme-linked immunosorbent assay (cELISA) was by the manufacture's manual with its kit (Equine infectious anemia virus antibody test kit, IDEXX, USA). In brief, One-hundred μl of each serum sample was place into the appropriate wells. One-hundred μl of the negative control and 100 μl of positive control were placed into their respective wells. Fifty μl of EIAV antigen conjugate was added into all the wells. The solution was mixed thoroughly by gently tapping the well holder 10 times, and incubated uncovered for 30 min at 37°C. The wells were washed by hand. One-hundred μl of TMB substrate solution was dispensed into each well, and the solution was mixed thoroughly by gently tapping the holder 10 times. The plate was incubated for 15 min at room temperature. One-hundred μl of stop solution was dispensed into each well to stop the reaction.

Test results were determined by a visual comparison of the sample color intensity as compared to the positive control (substrate has turned light blue) and negative control (substrate has turned dark blue).

We performed the survey on antibody against EIA among 10,040 animals (1,329 Jeju Ponies, 8,324 Jeju Pony-Crossbreds and 387 Thoroughbred horses) using cELISA. This survey found that no serological evidence of EIA presence in Jeju island for 7 years. Table 1 shows the history of serological test of EIA in Jeju, Korea.

EIAV disease in horses is apparently related to an exclusive infection of monocytes and macrophages, making EIA a relevant model for studying lentiviral pathogenicity from macrophage infections without the complications of lymphocyte infections associated with the immunodeficiency lentiviruses. In the present study, we detected serologically the

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Table 1. History of serological test of EIA in Jeju, Korea

Year	No. of samples	No. of positive	Test
1985*	161	0	IDT‡
2007-2009†	642	0	IDT‡
	Jeju Ponies 1,329	0	cELISA
2005-2011	Jeju Pony-Crossbreds 8,324	0	cELISA
	Thoroughbred horses 387	0	cELISA

*Rhee *et al*. 1986, †Yeh *et al*. 2011, ‡Immunodiffusion test

presence if EIA in horses.

Seropositivity is a good indication of infection because horses infected with the EIA virus carry it for life (2), with the development of a sustained antibody response appearing usually within 45 days of viral infection (3). The agar gel immunodiffusion test (AGID) (3), prescribed by the Office International des Épizooties (OIE) for international trade (7), is currently used in Canada for its EIA control program.

This test, specific to the p26 core viral protein, is relatively rapid, inexpensive, simple, and highly specific to identify animals infected with the EIA virus, although it is interpreted subjectively by visual reading of precipitation line curvature.

During the last few years, the detection of EIA antibodies by ELISA has been described and used in some countries where this test is commercialized under various formats. In the United States (USA), a few ELISAs have been approved by the US Department of Agriculture Animal and Plant Health Inspection Service in the 1990's as equivalent test methods for the diagnosis of EIA. Validation studies have indicated excellent agreement between these ELISAs and the AGID assay (1), with the ELISA being found, in some cases, to be even more sensitive than the AGID (11).

Jeju Ponies and Jeju Pony-Crossbreds were foaled and raised in Jeju Island. Thoroughbred horses are foreign-bred. The foreign-bred of any horses are imported after the national quarantine completely. Even Thoroughbred horses are free-EIA, the horses have been foaled by the breeders in the island since the 1980s. There was no report on outbreak the disease.

Previously, Rhee *et al*. reported that there was no Jeju horse among 161 horses with antibody against EIA by the immunediffusion test in 1985 (8). Recently, the survey found that no serological evidence of EIA presence among 642 horses in Jeju from 2007 to 2009 (13). However, Lee *et al*. have reported 3 horses with EIA from foreign countries from 2003 to 2008, when the horses had were quarantine in Korea (5). Fortunately, the area surrounded by the sea, so it was natural barrier from the equine pathogens.

There has been no horse with antibody against EIA in Jeju since 1985. We performed the large serological survey of EIA in Jeju from 2005 through 2011. Using the cELISA, a total of 10,040 animals (1,329 Jeju Ponies, 8,324 Jeju Pony-Crossbreds and 387 Thoroughbred horses) were tested at Equine Hospital of Jeju Race Park or Jeju Stud Farm. This survey found no serological evidence of EIA presence in Jeju. Consequently, surveillance conducted found no evidence of EIA activity.

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References

1. Burki F, Rossmanith W, Rossmanith E. Equine lentivirus, comparative studies on four serological tests for the diagnosis of equine infectious anemia. Vet Microbiol 1992; 33: 353-360.
2. Cheevers WP, McGuire TC. Equine infectious anemia virus: immunopathogenesis and persistence. Rev Infect Dis 1985; 7: 83-87.
3. Coggins L, Norcross NL, Nusbaum SR. Diagnosis of equine infectious anemia by immunodiffusion test. Am J Vet Res 1972; 33: 11-18.
4. Craigo JK, Montelaro RC. Encyclopedia of Virology, 3rd ed. Waltham: Academic Press 2008: 167-174.
5. Lee SK, Han JI, Yun SJ, Kang HG. A survey of epidemic diseases in horses imported into South Korea between 2003 and 2008. J Vet Clin 2010; 27: 268-272.
6. Oaks JL, McGuire TC, Ulibarri C, Crawford TB. Equine infectious anemia virus is found in tissue macrophages during subclinical infection. J Virol 1998; 72: 7263-7269.
7. OIE. Manual of Standards for Diagnostic Tests and Vaccines. France. 2000: 542-545.
8. Rhee YO, An SH, Jeon Y, Yoon YD, Park BK, Heo Y, Kim JM, Jang H, Kim YH, Sul DS, Song JB, Jung JK, Lee KH, Kim HP. The 1985 survey on horse diseases of veterinary importance in Korea. Korean J Vet Res 1986; 26: 87-92.
9. Sellon DC. Equine infectious anemia. Vet Clin North Am Equine Pract. 1993; 9: 321-336.
10. Sellon DC, Long MT. Equine infectious disease, 1st ed. Missouri: Saunders. 2007: 213-219.
11. Soutullo A, Verwimp V, Riveros M, Pauli R, Tonarelli G. Design and validation of an ELISA for equine infectious anemia (EIA) diagnosis using synthetic peptides. Vet Microbiol 2001; 79: 111-121.
12. Sponseller BA, Sparks WO, Wannemuehler Y, Li Y, Antons AK, Oaks JL, Carpenter S. Immune selection of equine infectious anemia virus *env* variants during the long-term inapparent stage of disease. Virology 2007; 363: 156-165
13. Yeh JY, Lee JH, Park JY, Cho IS, Choi YG, Yang SJ, Kyoung SG, Ahn KM, Kim HP, Moon JS. Serological survey for antibodies against equine infectious anemia virus in Republic of Korea from 2007 to 2009. Kor J Publ Hlyh 2011; 35: 195-198.
14. Zhang W, Lonning SM, McGuire TC. Gag protein epitopes recognized by ELA-A-restricted cytotoxic T lymphocytes from horses with long-term equine infectious anemia virus infection. J Virol 1998; 72: 9612-9620.

제주 사육 말에서 말전염성빈혈 조사

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요 약 : 말전염성빈혈(EIA)은 전세계의 마과동물에서 발생하는 전염병이다. 2005년부터 2011년까지 제주에서 사육되는 말을 대상으로 EIA를 조사하였다. 한국마사회 제주경마공원 동물병원에서 제주마 1,329 마리 및 한라마(제주산마) 8,324 마리 그리고 제주목장의 동물병원에서 Thoroughbred 말 387 마리 등 7년간 총 10,040마리를 효소면역측정법을 이용하여 검사하였다. 조사기간 동안 혈액에서 EIA 항체는 검출되지 않았다. 1985년 이후부터 제주에서 EIA 항체가 검출된 적이 없고, 발병 예도 없었다. 그러므로 제주에는 EIA가 없는 것으로 생각된다.

주요어 : 말전염성빈혈, 유병률, 제주, 제주마, Thoroughbred 말