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# A Survey of Epidemic Diseases in Horses Imported into South Korea between 2003 and 2008

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**Abstract :** South Korea is susceptible to foreign diseases due to its high rate of livestock importation. The purpose of this study was to investigate the infectious conditions of contagious disease of horses imported into South Korea from other countries. The horses were tested for contagious equine metritis (CEM), equine infectious anemia (EIA), equine piroplasmosis (EP), equine viral arteritis (EVA), vesicular stomatitis (VS), dourine, and glanders. The prevalence of these infectious diseases in 6,650 horses imported from 24 countries between 2003 and 2008 was reviewed by the National Veterinary Research and Quarantine Service. Seropositive results were found for EIA, EP, EVA, dourine and glanders: 3/6,189 serum samples tested were EIA-positive, 37/6,005 samples tested by complement fixation (CF) were EP-positive, 28/6,043 samples tested by virus neutralization (VN) were EVA-positive, 4/2,071 serum samples tested by CF were positive for dourine, and 4/1,950 samples tested by CF were positive for glanders. No contagious equine metritis or vesicular stomatitis was detected. In total, 76/6,650 imported horses tested positive for an infectious disease. Notably, 4/6 sera (66.6%), all taken from horses imported from Tanzania, were positive for glanders. This is the first report of glanders infection in horses from Tanzania since 1996.

***Key words*** **:** seroprevalence; horse; quarantine.

## Introduction

South Korea is dependent upon the importation of horses for breeding. It is, therefore, assumed that horses native to South Korea are susceptible to foreign diseases due to this high rate of livestock importation.

Horses imported into South Korea are quarantined and tested for contagious equine metritis, equine infectious anemia, equine piroplasma, dourine, vesicular stomatitis, glanders, and equine viral arteritis, in accordance with the regulations and relevant provisions of the Ordinance of the Ministry of Agriculture and Forestry in South Korea. It is noted that horses in quarantine may have already passed the relevant tests for some of these diseases under the conditions set by their exporting countries. A survey was undertaken in 1985 to investigate the health status of horses in South Korea using serological tests. This survey looked at 18 infectious diseases, including those outlined above, and reported that South Korea was free from dourine, glanders, vesicular stomatitis, equine piroplasma, equine viral arteritis, and contagious equine metritis (11). However, the importation of horses is still likely to be one of the main sources of infectious disease in South Korea.

Test results for infectious diseases in recently imported horses would be useful for producing up-to-date quarantine policies in South Korea. A review of the diagnostic tests used to screen for these diseases, and the validity of these tests, may lead to more up-to-date methods being available to the National Veterinary Research and Quarantine Service. However, to date, no survey has been conducted into the prevalence of infectious disease in horses imported into South Korea.

Here, we report the results of laboratory tests for seven infectious diseases in horses imported between 2003 and 2008.

## Materials and Methods

### Sample collection

Blood samples drawn from the jugular vein were tested for equine infectious anemia, equine piroplasma, dourine, vesicular stomatitis, glanders, and equine viral arteritis. Urogenital swabs taken from both mares and stallions were used to test for contagious equine metritis.

### Contagious equine metritis (CEM)

A positive control for CEM was donated by the Institute for Animal Science and Health, ID-DLO, Netherlands.

Diagnostic test was carried out as described in OIE terrestrial manual (4). Briefly, the test swabs were inoculated into Timoney's medium (7% sheep blood added to Eugon agar;

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Difco™, Franklin Lakes, NJ, USA) (13), a selective media for *Taylorella equigenitalis*, and cultured in an atmosphere of 5% $CO_2$ for 6 days. Colonies were selected on the second, fourth, and sixth day and cultured for 48 hours in chocolate medium in the absence of antibiotics. Colonies showing the same biochemical activity as the positive control were identified using an antibody agglutination kit (Mono-tayl®, Bionor Co., Skien, Norway).

**Equine infectious anemia (EIA)**

EIA was diagnosed using the ViraCHEK®/EIA kit (Synbiotics Corp, Kansas city, MO, USA) according to the manufacturer's instructions. A positive result was indicated by the formation of precipitation lines between the sera and the standard reagent.

**Equine piroplasma (EP)**

'Standard' samples of *Babesia equi* and *Theileria equi* were donated by the National Veterinary Services Laboratory (USDA; Iowa, USA). A complement fixation (CF) test was used to test for EP, and a titer > 1:4 was regarded as positive referred to OIE terrestrial manual (7).

**Equine viral arteritis (EVA)**

The EVA virus Bucyrus strain was donated by the National Veterinary Services Laboratory (USDA; Iowa, USA) and was used as a positive control. The samples were tested by Virus Neutralization (VN). When the titer of the serum inhibiting the cytopathic effect (CPE) was > 1:4, the result was considered positive as described previously (8).

**Vesicular stomatitis (VS)**

The standard strains, antiserum of VS virus-NJ, and VS virus-IND were donated by the National Veterinary Services Laboratory (USDA; Iowa, USA). A competitive enzyme-linked immunosorbent assay (C-ELISA) was used for the identification of infectious agents according to OIE terrestrial manual (10). A procedure described previously was used for the test of VS (10).

**Dourine and Glanders**

The standard agents and antiserum were donated by the National Veterinary Services Laboratory (USDA; Iowa, USA) and used in the CF tests. The CF tests were conducted for each infectious agent, according to OIE terrestrial Manual (5,9). Procedures described previously were used for the test of Dourine and Glanders (5,9).

**Table 1.** Statistics of imported horses into South Korea from 2003 to 2008

| Year | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | Total |
|---|---|---|---|---|---|---|---|
| No. of head | 1,199 | 1,589 | 1,151 | 1,035 | 910 | 766 | 6,650 |

**Table 2.** Seropositive rate of infectious diseases in horses imported into South Korea between 2003 and 2008

| Country | No. of head | | CEM | EIA | EP | EVA | VS | Dourine | Glanders |
|---|---|---|---|---|---|---|---|---|---|
| Australia | 1,217 | No. of samples | 911 | 1,217 | 1,217 | 972 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| Belgium | 80 | No. of samples | 20 | 74 | 67 | 73 | - | 23 | 23 |
| | | Positive (%) | - | - | - | 2 (2.7) | - | - | - |
| Brazil | 25 | No. of samples | 5 | 24 | 25 | 24 | 24 | 24 | - |
| | | Positive (%) | - | - | 9 (36.0) | - | - | - | - |
| China | 2,331 | No. of samples | 1,549 | 1,938 | 19,17 | 1,991 | - | 1,902 | 1,802 |
| | | Positive (%) | - | 3 (0.2) | 14 (0.7) | 8 (0.4) | - | 4 (0.2) | - |
| Denmark | 3 | No. of samples | - | 3 | 3 | 3 | - | 3 | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| France | 28 | No. of samples | 28 | 28 | 11 | 28 | - | 25 | 25 |
| | | Positive (%) | - | - | - | - | - | - | - |
| Germany | 471 | No. of samples | 130 | 450 | 449 | 470 | - | - | - |
| | | Positive (%) | - | - | - | 4 (0.9) | - | - | - |
| Hungary | 6 | No. of samples | 2 | 6 | 6 | 6 | - | 6 | 6 |
| | | Positive (%) | - | - | - | - | - | - | - |
| India | 12 | No. of samples | 4 | 12 | 12 | 12 | - | 6 | 12 |
| | | Positive (%) | - | - | - | - | - | - | - |
| Ireland | 10 | No. of samples | 6 | 6 | 6 | 3 | - | 3 | 3 |
| | | Positive (%) | - | - | - | - | - | - | - |

**Table 2.** Continued

| Country | No. of head | | CEM | EIA | EP | EVA | VS | Dourine | Glanders |
|---|---|---|---|---|---|---|---|---|---|
| Japan | 322 | No. of samples | 300 | 318 | 318 | 321 | - | 18 | 18 |
| | | Positive (%) | - | - | - | - | - | - | - |
| Kazakhstan | 18 | No. of samples | 12 | 15 | 15 | 15 | - | 12 | 12 |
| | | Positive (%) | - | - | 9 (60) | - | - | - | - |
| North Korea | 5 | No. of samples | 2 | 5 | 5 | 5 | - | 5 | 5 |
| | | Positive (%) | - | - | - | - | - | - | - |
| Malaysia | 9 | No. of samples | 2 | 9 | 9 | 9 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| Netherlands | 5 | No. of samples | 3 | 5 | 5 | 5 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| New Zealand | 153 | No. of samples | 1 | 151 | 12 | 153 | - | - | - |
| | | Positive (%) | - | - | - | 1 (0.7) | - | - | - |
| Qatar | 8 | No. of samples | 1 | 8 | 8 | 8 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| Russian | 38 | No. of samples | 6 | 38 | 38 | 38 | - | 38 | 38 |
| | | Positive (%) | - | - | - | 12 (31.6) | - | - | - |
| Singapore | 2 | No. of samples | 2 | 2 | 2 | 2 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| Spain | 2 | No. of samples | 1 | 2 | 2 | 2 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| Tanzania | 6 | No. of samples | 6 | 6 | 6 | 6 | - | 6 | 6 |
| | | Positive (%) | - | - | 5 (83.3) | - | - | - | 4 (66.7) |
| Thailand | 2 | No. of samples | 1 | 2 | 2 | 2 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| UK | 12 | No. of samples | 11 | 12 | 12 | 12 | - | - | - |
| | | Positive (%) | - | - | - | - | - | - | - |
| USA | 1,885 | No. of samples | 1,673 | 1,858 | 1,858 | 1,883 | 1,858 | - | - |
| | | Positive (%) | - | - | - | 1 (0.1) | - | - | - |
| Total | 6,650 | No. of samples | 4,676 | 6,189 | 6,005 | 6,043 | 1,882 | 2,071 | 1,950 |
| | | Positive (%) | 0 | 3 | 37 | 28 | 0 | 4 | 4 |

CEM: contagious equine metritis, EIA: equine infectious anemia, EP: equine piroplasma,
EVA: equine viral arteritis, VS: vesicular stomatitis.

## Results

The numbers and country of origin of horses imported into South Korea between 2003 and 2008 are shown in Table 1. The table shows that 6,650 horses from 24 countries were imported during this period. The main exporters were China (2,331 horses; 35.1%), USA (1,885 horses; 28.3%), Australia (1,217 horses; 18.3%), and Germany (471 horses; 7.1%).

While in quarantine, 37/6,005 horses tested positive for EP, 28/6,043 for EVA, 4/2,071 for dourine, 4/1,950 for glanders, and 3/6,189 for EIA: a total of 76 animals. All horses tested negative for CEM and VS (Table 2). Notably, 4/6 (66.6%) of the sera taken from horses imported from Tanzania were positive for glanders.

## Discussion

CEM is a venereal disease of horses and is endemic in Europe (12). Only horses proven to be free of CEM for the previous 6 months are allowed into South Korea in accordance with the regulations and relevant provisions of the Ordinance of the Ministry of Agriculture and Forestry, which governs such importations. No horses were found to be positive for CEM in this study. However, antibodies to CEM are undetectable by 2-3 weeks after recovery from CEM infection (2), and so serological testing is unsuitable for quarantined animals. Diagnosis can only be made by isolation of the agent from genital tract swabs followed by culture of the infectious agent; a method endorsed by the World Organization for Ani-

mal Health (OIE). However, polymerase chain reaction (PCR) is used for the detection of *T. equigenitalis* in the Netherlands, Japan, and the UK (4). Isolation of the agent by culture of genital swabs was the method used in our study. PCR has the advantage of speed and the ability to detect very low levels of infectious agent (4), and it is recommended that the quarantine service in South Korea substitute culture methods with PCR-based methods.

EIA is endemic in many parts if the world (8). In 1985, EIA was reported in South Korea, but the number of positive cases was low (11). The number of positive EIA-positive cases in our study was also low: 1/5,179 horses. However, although the number of positive cases is low, screening for EIA is still recommended as treatment is not possible, and a commercial vaccine is not yet widely available (6).

EP is reported to be endemic in many countries, except the USA, Canada, Australia, Japan, England, Iceland, Ireland, and South Korea (1,11). Nine horses from Brazil (36%), 14 horses from China (0.7%), 9 horses from Kazakhstan (60%), and 5 horses from Tanzania (83.3%) tested positive for EP in our study. The number of positive cases in horses imported from Brazil, Kazakhstan and Tanzania was high in percentage terms, strongly suggesting that EP is prevalent in these countries. Because of this, horses imported from these countries should be stringently tested for EP while in quarantine. The CF test is the standard laboratory test for diagnosing infection with EP agents, but false positive results do occur (7).The OIE currently prefers the indirect fluorescent antibody (IFA) test and the competitive enzyme-linked immunosorbent assay (C-ELISA) for the detection of EP, rather than the CF test (7). However, the CF test is still used for the detection of EP by the quarantine service in South Korea at this time. We suggest that the CF test should be replaced with either the IFA or C-ELISA.

EVA infection is assumed to be endemic worldwide (3). In this study, two horses from Belgium, 8 horses from China, 4 horses from Germany, 1 horse from New Zealand, and 12 horses from Russia tested positive for EVA. All horses should be free of EVA or not vaccinated before importation of into South Korea and, only stallions may be allowed to be vaccinated against EVA before importation into South Korea in accordance with the regulations and relevant provisions of the Ordinance of the Ministry of Agriculture and Forestry. No stallions tested positive in this study and so, consequently, the positive results obtained mean EP infection.

While we were undertaking this survey, VS infection was confirmed in the USA and Brazil (14). None of the horses in our survey tested positive for VS. The OIE recommends the CF test, the Virus Neutralization (VN) test, and ELISA as the diagnostic tests meeting the requirements for international horse transport and trade. C-ELISA was used in our study, and has the advantage of longer antibody persisting time than the CF test (10).

Dourine has been in decline in many countries throughout the 20$^{th}$ century, but the disease is present in Russia, Namibia, Lesotho, and Botswana. The number of positive dourine cases in our study (diagnosed using the CF test) was low, and all occurred in horses imported from China.

We detected glanders [found in the Middle East, Asia, Africa, and South America (9)] in a high number of horses imported from Tanzania. No known glanders cases have been reported in Tanzania since 1996. Our study is the first report confirming glanders in horses from Tanzania since that time. As glanders is a zoonosis, intensified quarantine is recommended.

The disqualification rate for horses quarantined from Tanzania, Kazakhstan and Brazil was very high in percentage terms. It is assumed that this is due to differences in disease occurrence, and the levels and effectiveness of disease control in these countries.

This is the first quarantine survey of infectious diseases present in horses imported into South Korea. There has been no survey of horse diseases in South Korea for the last 20 years. This survey will lead to improvements in the testing methods used in the current quarantine system.

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# 2003년부터 2008년까지 한국에 수입된 말에서 전염성 질병의 실태조사

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**요 약** : 한국은 가축의 수입이 많아 외래성 질병에 항상 노출되어 있다. 이에 본 연구의 목적은 2003년부터 2008년까지 외국에서 한국으로 수입된 말의 전염성 질환의 발생실태를 조사하기 위하여 실시하였다. 2003년부터 2008년까지 수입된 6,650두의 말을 대상으로 가축전염병예방법에 등재되어 있는 주요 전염병 7개 항목(말전염성빈혈, 말파이로플라스마병, 말전염성동맥염, 비저, 구역, 전염성자궁염, 수포성구내염)을 검사하였다. 말전염성빈혈은 수입된 말 6,189두 중 3두에서 양성을 나타내었으며, 말파이로플라스마병은 6,005두 중 37두, 말전염성동맥염은 6,043두 중 28두, 구역은 2,071두 중 4두, 비저는 1,950두 중 4두가 양성을 나타내었다. 말전염성자궁염과 수포성구내염은 모든 말에서 검출되지 않았다. 전체적으로 수입된 말 6,650두 중 76두가 전염성 질환에 대해 양성을 나타내었다. 특히 탄자니아에서 수입된 말의 66.6%가 비저에 양성을 나타내었다는 것은 주목할만한 결과이며, 이것은 1996년 이래 최초로 검출되었다.

**주요어** : 혈청학적 검사, 말, 검역