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Epidemiological investigation of equine hindlimb ataxia with *Setaria digitata* in South Korea

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ABSTRACT

Background: Since 2013, the number of requests for diagnosis for horses based on neurological symptoms has increased rapidly in South Korea. The affected horses have commonly exhibited symptoms of acute seasonal hindlimb ataxia. A previous study from 2015–2016 identified *Setaria digitata* as the causative agent.

Objectives: This study is an epidemiological investigation to find out risk factors related to the rapid increase in hindlimb ataxia of horses due to aberrant parasites in South Korea. **Methods:** An epidemiological investigation was conducted on 155 cohabiting horses in 41 horse ranches where the disease occurred. The surrounding environment was investigated at the disease-causing horse ranches (n = 41) and 20, randomly selected, non-infected ranches. **Results:** Hindlimb ataxia was confirmed in nine cohabiting horses; this was presumed to be caused by ectopic parasitism. Environments that mosquitoes inhabit, such as paddy fields within 2 km and less than 0.5 km from a river, had the greatest association with disease occurrence. **Conclusions:** Most horse ranches in South Korea are situated in favorable environments for mosquitoes. Moreover, the number of mosquitoes in the country has increased since 2013 due to climate change. Additional research is required; however, these data show that it is necessary to establish guidelines for the use of anthelmintic agents based on local factors in South Korea and disinfection of the environment to prevent disease outbreaks.

Keywords: Epidemiology; ataxia; horses; mosquito vectors; setaria nematode

INTRODUCTION

According to the Animal and Plant Quarantine Agency of South Korea, there were 16 requests for diagnosis based on horse neurological symptoms between 1987 and 2012. By contrast, the number of diagnosis requests has increased since 2013; 11 and 19 cases were reported in 2013 and 2014, respectively. Most of the affected horses showed symptoms of acute hindlimb ataxia; therefore, a study was conducted to identify its cause in horses of South



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Conflict of Interest

The authors declare no conflicts of interest.

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S. digitata is a parasite of cattle and hoofed animals and is found mainly in Asia [2,3]. The intermediate hosts are mosquitoes, such as *Anopheles sinensis (A. sinensis), Armigeres subalbatus (A. subalbatus)*, and *Aedes togoi (A. togoi)* [4]. The adult worms are parasitic in the peritoneum and pleural cavity of the final hosts. They can cause mild fibrinous peritonitis but are generally non-pathogenic in their natural hosts [5]. However, infection into an aberrant host can lead to the *S. digitata* entering the nervous system, which can result in severe neurological symptoms [3,5]. The neurological symptoms caused by aberrant parasites in horses mainly appear on the hind legs [3]. Most infected horses have a weak response to treatment; although there may be improvement in symptoms, they often show permanent functional abnormalities. Cerebrospinal nematodiasis has fatal results for racehorses or horses whose main purpose is exercise.

In this study, epidemiological investigations were conducted to identify the cause of the rapid increase in hindlimb ataxia of horses due to aberrant parasites in South Korea; and to assess the association between risk factors and the occurrence of disease. It was hypothesized that proximity to the environment in which mosquitoes (the intermediate host of *S. digitata*) are prevalent and to cattle (a definitive host) are associated with an increased risk of hindlimb ataxia in horses.

MATERIALS AND METHODS

Physical and gait examinations were performed on 155 horses living together on ranches where the hindlimb ataxia had occurred between 2015 and 2016. Biological samples (blood, feces, and nasal swab) were collected to test for infectious agents. PCR was performed on whole blood and nasal swabs to test for neurotropic viruses [1]. Whole blood PCR was performed to detect the following neurotropic viruses: equine herpesvirus-1 & -4 (EHV-1, EHV-4), equine influenza virus (EIV), Japanese encephalitis virus, and West Nile virus (WNV). Nasal swab PCR was performed to detect EHV-1, EHV-4, and EIV. A neutralization test of serum samples, or enzyme-linked immunosorbent assay, was performed to detect the presence of antibodies for EHV-1 and EHV-4. A fecal flotation test was performed to detect the eggs of parasites.

An epidemiological study was conducted to identify the associations between environmental variables and the outbreak of hindlimb ataxia of horses. Forty-one horse ranches where horses with symptoms of hindlimb ataxia were reported from 2015–2016 were included in this study. Control ranches were chosen by randomly selecting 20 horse ranches from a list of all ranches without outbreaks of equine hindlimb ataxia in South Korea. Data were collected by investigating the surrounding environments of all 61 horse ranches for two years, from 2015–2016, using Naver Map and Kakao Map. The potential associations between environmental factors and the outbreak of hindlimb ataxia in horses was considered. The environmental factors considered were < 0.5 km from a river; close proximity to mountains and/or the city; existence of cattle ranches in a 2 km vicinity; and the number of paddy fields, reservoirs, ponds, or presence of a fishing pond within 2 km. Variables that were highly



related to the outcome (p < 0.2) in the univariable analysis were included in the multivariable logistic regression. All statistical analyses were conducted using R version 4.0.4. The values of p < 0.05 were considered significant for these final analyses.

RESULTS

Physical examination of 155 horses cohabiting with the affected individuals found an additional nine horses with hindlimb ataxia. These horses were not necropsied because the owner did not want further examination.

Virus infection could be excluded because whole blood and nasal swab PCR were negative in all samples (**Table 1**). The equine herpesvirus neutralizing antibody titers varied more than 256-fold from negative. In the fecal flotation test, the eggs of strongyles were confirmed in eight diseased horses, and various parasite eggs were identified in the cohabiting horses (**Table 1**). The parasite eggs of *Strongylus spp.* were confirmed in the feces of 26 cohabiting horses, eggs of *Parascaris equorum* in five horses, eggs of *Oxyuris equi* in one horse, eggs of *Dictyocaulus arnfieldi* in six horses, eggs of *Eimeria leuckarti* in one horse, and larvae in three horses to varying degrees (**Table 1**). One horse ranch that had three horses with neurological symptoms in 2015 also had one horse with symptoms in 2016. One notable point is that about half of the horses living together on this ranch had parasite eggs in the feces.

Data were collected for 41 horse ranches with cases and 20 control horse ranches. All environmental factors were screened for univariable association with the outbreak of equine hindlimb ataxia. Variables that were associated with the outcome (p < 0.2) in the univariable analysis and considered for inclusion in the multivariable model were: existence of cattle ranches in a 2 km vicinity, number of paddy fields in a 2 km vicinity, < 0.5 km distance from a river, and proximity to the city (**Table 2**). The multivariable logistic regression model is shown

Table 1. The test results of the neurotropic viruses and parasites on 50 diseased and 155 cohabiting horses

Tests	Viruses and parasites	Diseased horses	Cohabitating horses
Blood PCR	EHV-1	-	-
	EHV-4	-	-
	EIV	-	-
	JEV	-	-
	WNV	-	-
Nasal swab PCR	EHV-1	-	-
	EHV-4	-	-
	EIV	-	-
SN titer or ELISA	EHV-1	Variableª	Variableª
	EHV-4	Variableª	Variableª
Parasite eggs	Strongyles	8	26
	Parelaphostrongylus tenuis	-	-
	Drachia megastoma	-	-
	Habronema microstoma	-	-
	Parascaris equorum	-	5
	Oxyuris equi	-	1
	Dictyocaulus arnfieldi	-	6
	Eimeria leuckarti	-	1
	Larvae	-	3

PCR, polymerase chain reaction; EHV-1, equine herpesvirus-1; EHV-4, equine herpesvirus-4; EIV, equine influenza virus; JEV, Japanese encephalitis virus; WNV, West Nile virus; SN, serum neutralization; ELISA, enzyme-linked immunosorbent assay.

^aNegative to > 256.



Table 2. Univariable analysis of environmental risk factors for hindlimb ataxia of hor	ses from South Korea
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Variables			Univarial	ole analysis	
	Coefficient	Standard error	Odds ratio	95% confidence interval	p-value (likelihood ratio)
Existence of cattle ranches in a 2 km vicini	ty				0.005ª
No	Ref.				
Yes	1.764	0.629	5.83	1.7-20	
# of paddy fields in a 2 km vicinity					0.003ª
< 2	Ref.				
≥ 2	1.75	0.593	5.76	1.8-18.42	
< 0.5 km from a river					0.011ª
No	Ref.				
Yes	1.545	0.608	4.69	1.42-15.42	
Mountainous terrain					0.412
No	Ref.				
Yes	-0.454	0.553	0.63	0.21-1.88	
City					0.057ª
No	Ref.				
Yes	-1.175	0.617	0.31	0.09-1.03	
# of reservoirs in a 2 km vicinity					0.300
< 2	Ref.				
≥ 2	-0.598	0.577	0.55	0.18-1.7	
# of ponds in a 2 km vicinity					0.529
< 2	Ref.				
≥ 2	-0.345	0.548	0.71	0.24-2.07	
Existence of fishing ponds in a 2 km vicinity					0.596
No	Ref.				
Yes	-0.377	0.713	0.69	0.17-2.77	
		0.713		0.17-2.77	

^ap < 0.2 in the univariable analysis were then considered for inclusion in a multivariable regression model.

Table 3. Multivariable logistic regression model of environmental risk factors for hindlimb ataxia of horses from South Korea

Multivariable analysis				
Coefficient	Standard error	Odds ratio	95% confidence interval	p-value (likelihood ratio)
				0.549
Ref.				
0.513	0.845	1.67	0.32-8.76	
				0.041ª
1.587	0.799	4.89	1.03-23.43	
				0.01 ^a
1.733	0.722	5.66	1.38-23.29	
				0.408
-0.644	0.772	0.53	0.12-2.39	
	Ref. 0.513 1.587 1.733	Ref. 0.845 1.587 0.799 1.733 0.722	Coefficient Standard error Odds ratio Ref. 0.513 0.845 1.67 1.587 0.799 4.89 1.733 0.722 5.66	Coefficient Standard error Odds ratio 95% confidence interval Ref. 0.513 0.845 1.67 0.32-8.76 1.587 0.799 4.89 1.03-23.43 1.733 0.722 5.66 1.38-23.29

^ap < 0.05.

in **Table 3**. The number of paddy fields in a 2 km vicinity and < 0.5 km from a river were associated with an increased risk of hindlimb ataxia caused by aberrant parasites in horses.

DISCUSSION

Hindlimb ataxia in horses can have different causes. The causative agents can be broadly divided into infectious and non-infectious. In a previous study, it was confirmed that outbreaks of hindlimb ataxia in horses, which has been increasing in South Korea, are seasonal. Additionally, suppurative or granulomatous lesions, which are histological changes confirmed during bacterial infection, were not present in any of the 50 horses [1]. Therefore,



this epidemiological investigation focused on infectious agents, especially viruses and parasites, rather than non-infectious agents.

In this study, neurotropic virus infection was not confirmed in any of the 155 cohabiting horses. However, similar types of neurological symptoms were confirmed around the same time in nine cohabiting horses under the same environment. Neurotropic viruses were excluded; therefore, it can be assumed that these were caused by the aberrant migration of parasites, such as *S. digitata*. Therefore, this study evaluated the differences between horse ranches with and without parasite-induced neurological symptoms occurrence.

For *S. digitata* infection in horses to increase, there must be cattle nearby and a habitat for mosquitoes in the vicinity. The surrounding environment of the horse ranches where the horses had symptoms of hindlimb ataxia from 2015–2016 were near a cattle ranch (85.4%), within 2 km of paddy fields (75.6%), and < 0.5 km away from rivers (61%). Reservoirs, ponds, and fishing ponds were sometimes identified in the vicinity (**Table 4**). These factors were also investigated in disease-free ranches. Taken together, these analyses revealed two significant environmental factors associated with disease occurrence: the number of paddy fields in a 2 km vicinity and < 0.5 km from a river. An increased risk of parasite-induced equine hindlimb ataxia was associated with the number of paddy fields/fields within 2 km. Horse ranches with two or more paddy fields in a 2 km vicinity were five times more likely to have horses with hindlimb ataxia than horse ranches with less than two paddy fields in a 2 km vicinity. Additionally, the ranches situated < 0.5 km from a river were at increased risk; they were six times more likely to have horses with hindlimb ataxia than ranches that are > 0.5 km from a river.

S. digitata is transmitted by mosquitoes, including *Aedes* spp., *Culex* spp., and *Anopheles* spp. [4]. In central Japan, *A. sinensis* is the major vector of *S. digitata*. From 2012–2015, most

Variables 0	Case ranches (n = 41)	Control ranches (n = 20)	<i>p</i> -value	
Existence of cattle ranches in a 2 km vicinity				
No	6 (14.6)	10 (50.0)		
Yes	35 (85.4)	10 (50.0)		
# of paddy fields in a 2 km vicinity			0.005	
< 2	10 (24.4)	13 (65.0)		
≥ 2	31 (75.6)	7 (35.0)		
< 0.5 km from a river			0.018	
No	16 (39.0)	15 (75.0)		
Yes	25 (61.0)	5 (25.0)		
Mountainous terrain			0.582	
No	21 (51.2)	8 (40.0)		
Yes	20 (48.8)	12 (60.0)		
City			0.102	
No	34 (82.9)	12 (60.0)		
Yes	7 (17.1)	8 (40.0)		
# of reservoirs in a 2 km vicinity			0.454	
< 2	30 (73.2)	12 (60.0)		
≥ 2	11 (26.8)	8 (40.0)		
# of ponds in a 2 km vicinity			0.722	
< 2	24 (58.5)	10 (50.0)		
≥ 2	17 (41.5)	10 (50.0)		
Existence of fishing ponds in a 2 km vicin	ity		0.870	
No	35 (85.4)	16 (80.0)		
Yes	6 (14.6)	4 (20.0)		

 Table 4. Baseline characteristics of horse ranch environment

Values are presented as number (%).



mosquito species in South Korea were *Anopheles* spp., *Aedes* spp., or *Culex* spp. The species with the highest incidence in South Korea is A. sinensis, accounting for 50%-60%; Aedes have a prevalence of 20%–30% and *Culex* account for 5%–10% [6,7,8,9]. One of the causes of the increase in equine hindlimb ataxia from aberrant parasites between 2015 and 2016 in South Korea is hypothesized to be an increase in mosquito vectors. Therefore, the favorable environments for mosquitoes were investigated in this study. Anopheles lay eggs and inhabit large areas with clean water, such as paddy fields, waterways, and streams [10]. Therefore, they are most often found in city suburbs or rural areas from July to August [9,11,12]. Aedes are most prevalent from July to September [13,14] in forests, palace gardens, mountains, and green areas within cities [15]. Culex have a similar habitat to Anopheles; therefore, they have a relatively high population density in rural areas [15]. Their prevalence is high from July to September [10,11]: however, mid-September has been reported more recently as the highest population density due to the prolonged temperature increase after August [7,8,9]. The national annual incidence of Anopheles has recently increased rapidly, from 1.6-4.0× higher between 2013 and 2015 [6,7,8,9]. In addition, Aedes has shown a 3.3× higher incidence in 2016 than in 2013 [14.16]. Horse ranches are located near mosquito habitats in South Korea and the mosquito population has increased due to temperature increases and changes in precipitation since 2013; therefore, it can be theorized that parasitic infection has increased. This has led to an increase in horses exhibiting the symptoms of hindlimb ataxia.

Authors have hypothesized that the presence of cattle, a definitive host, in the vicinity of a horse ranch would have a significant relationship with disease onset. However, this study confirmed that proximity of a mosquito habitat, and not cattle, was significantly associated with equine hindlimb ataxia. Nonetheless, 85% of the horse ranches where disease occurred were near a cattle ranch. In the future, a full-scale investigation of domestic cattle should be conducted to determine the *S. digitata* infection rate. Furthermore, this could confirm the status and effectiveness of anthelmintic agents for cattle.

Hindlimb ataxia caused by *S. digitata* occurred even though most horses were receiving anthelmintic agents through a support project from the Korea Racing Authority at the time of the study. In addition, 50% of cohabitating horses on a ranch with disease outbreaks for two consecutive years had parasite eggs in their feces. It can be theorized that the risk of developing neurological symptoms due to ectopic parasitism will increase in horse ranches that are not well controlled for parasites. The possibility of ineffective anthelmintic agents or poor timing of administration should be considered. Resistance may have developed over time due to the consistent use of the same formulation. Effectiveness will decrease because the anthelmintic agents are administered at regular intervals without considering the mosquito outbreak period and parasite life cycle. Further research is required on the current status and effectiveness of anthelmintic agents in horses in South Korea.

This is the first epidemiological investigation of the environmental risk factors for the outbreak of equine hindlimb ataxia caused by parasites. This study provides owners and individuals associated with the horse industry with information on horse ranches and the environmental factors that are more likely to have equine hindlimb ataxia outbreaks. Additionally, these data support the need for these ranches to be managed and improved to reduce outbreaks. The factors that significantly increased the risk of hindlimb ataxia in horses were the number of paddy fields in a 2 km vicinity and rivers < 0.5 km from ranches. It is not easy to choose a location for a horse ranch that avoids paddy fields and rivers in South Korea. However, parasite infection can be avoided by keeping the environment clean, disinfecting the



surroundings, and following the guidelines for anthelmintic treatment that consider mosquito outbreak periods. Hindlimb ataxia in horses caused by *S. digitata* can be reduced if horses are raised in locations that avoid cattle ranches, or if the infection rate is determined through a full-scale investigation of domestic cattle and deworming is performed.

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