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Retrospective study on equine viral abortions in Poland between 1999 and 2022

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Abstract

Introduction: Loss of pregnancy in mares is a major cause of economic and emotional impact for horse breeders. It can have many different infectious and noninfectious causes. The aim of this study was identification of the main viral causes of abortion in mares in Poland based on tissue samples from 180 aborted foetuses submitted for testing between 1999 and 2022. **Material and Methods:** Tissues of aborted foetuses collected from different horse studs throughout Poland were tested for the presence of equine herpesvirus types 1 and 4 (EHV-1/-4) and if negative, for equine arteritis virus (EAV). The examination was performed using a PCR/reverse transcriptase PCR (1999–2012) and a quantitative PCR (2013–2022). **Results:** The cause of abortion was determined to be EHV-1 in 49.4% of cases (n = 89), whereas no EHV-4- or EAV-positive cases were found. The proportion of abortions due to EHV-1 differed between regions, with the highest percentage in the Lubelskie and Wielkopolskie provinces. **Conclusion:** The results of the study indicate that EHV-1 is the most important viral infectious agent causing abortions in mares in Poland.

Keywords: abortion, EHV-1/-4, EAV, PCR, quantitative PCR.

Introduction

Outbreaks of abortions in mares can have a significant economic impact on horse breeding. While abortions can be caused by environmental or genetic factors, in naïve studs the majority of them are connected with infectious agents including viruses (17). The most important viral agents that cause abortions in horses are equine arteritis virus (EAV), equine herpesvirus type 1 (EHV-1) and occasionally equine herpesvirus type 4 (EHV-4) (6).

Equine arteritis virus is a member of the *Alphaarterivirus* genus within the *Arteriviridae* family (18). Although infections with this virus often remain subclinical, they can also lead to the development of clinical signs including pyrexia, depression, anorexia, oedema, conjunctivitis, petechial haemorrhages on mucosal surfaces and urticaria. Infections in foals can lead to severe interstitial pneumonia or pneumoenteric syndrome (3). The virus can cause abortions in mares infected after two months of gestation. Infection in pregnant mares can cause myometritis with a degeneration of the myocytes and an infiltration of the mononuclear cells, and can lead to foetal death through decreased blood supply to the foetus (16). The pathogen also infiltrates foetal tissues, causing atrophy of the lymphoid follicles in the spleen and lymph nodes. The stress associated with the infection of the foetus could activate the foetal hypothalamic pituitary axis, representing another mechanism that might induce abortion (3). Oedematous changes and degeneration of fibroblasts in the subvillous layers of the placenta could be observed as a result of infection (32). Between approximately 10% and over 50% of infected pregnant mares abort within 10 to 33 days of infection with the virus during the acute or early convalescent stages (6, 16, 18). In Poland, a longitudinal study performed between 1976 and 2010 showed that EAV infections were associated with over 23% of cases of abortion and neonatal death in the analysed period (5).

Equine herpesvirus 1 and 4 are important respiratory pathogens in horses. Although many infections are subclinical, some can lead to serious economic losses (23). Both viruses are classified in the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, and *Varicellovirus* genus and are closely related genetically and antigenically. They manifest considerable cross reactivity (22). While EHV-4 causes moderate respiratory infection in young horses and sporadic abortions, EHV-1 is responsible for a respiratory disease of varying severity, abortions, neonatal foal deaths or a neurological disease called equine herpesvirus myeloencephalopathy (10).

Following primary infection with EHV-1, the virus establishes life-long latency within its host in trigeminal ganglia or in T lymphocytes (2, 24). It is generally believed that under specific circumstances such as stressful conditions (travelling, rehousing, participation in competitive events, unsettled social structures or changes in the daily routine) the virus can periodically reactivate from latency, which may or may not be accompanied by disease (23).

Diagnosis of EHV-1, -4 and EAV infections is commonly based on PCR or quantitative PCR (qPCR) analysis of nasal swabs, blood, and/or tissues. Serological assays, such as ELISAs or virus neutralisation tests are also available. Paired (acute and convalescent) serum samples with significant increases in antibody titres can indicate recent infection. Early laboratory identification of specific viral DNA or RNA is crucial for veterinarians and horse breeders, as it allows rapid implementation of the most appropriate control measures. The variability of clinical features and many difficulties associated with the correct diagnosis of EHV-1, -4 or EAV infections make DNA or RNA identification particularly important. Therefore, each suspicion of EHV-1, -4 or EAV infection should be confirmed or disproved by laboratory assays performed in a specialised laboratory with qualified personnel and appropriate equipment.

The purpose of the study was to determine the main viral causes of abortion in mares in Poland between 1999 and 2022.

Material and Methods

Sample collection. In total, tissue samples from 180 aborted foetuses were collected between 1999 and 2022 from national horse studs and small, private stables located throughout Poland (Fig. 1). Samples were submitted to the Department of Virology of the National Veterinary Research Institute (NVRI) in Puławy by field veterinarians for laboratory investigation to identify the cause of abortion. The majority of pregnant mares in large national studs were treated in a routine yearly vaccination programme against equine rhinopneumonitis with a combined EHV-1/-4 preparation. No information on vaccinations in small, private studs was available. The collected foetal tissue samples included lung, liver and spleen sections. Additionally, in some submissions kidney, heart and placenta tissue were also included. All tissues were sent to the laboratory on ice packs and were typically received within 1–4 days of collection. When available, necropsy reports revealed that the abortions occurred during the last trimester of pregnancy, with or without any apparent gross lesions. Blood samples or nasal swabs collected from mares at the time of abortion were not available. Neither histological nor bacteriological investigation was attempted.

Sample preparation. On arrival at the NVRI, the samples were processed according to the laboratory protocols standard in the institute at the time of submission.

Fig. 1. Geographical distribution and number of cases of equine abortion in Poland 1999–2022 Brackets enclose the number of EHV-1 cases

Table 1. Primers and probes used for detection of equine herpesvirus 1 (EHV-1), equine herpesvirus 4 (EHV-4) and equine arteritis virus (EAV) between 1999 and 2022

Assay	Virus	Region	Primers / probes (5′ to 3′)	Size (bp)	Reference
Quantitative PCRs	EHV-1	gB	Forward: CATGTCAACGCACTCCCA	63	(9)
			Reverse: GGGTCGGGCGTTTCTGT		
			Probe: FAM-CCCTACGCTGCTCC-TAMRA		
	EHV-4		Forward: GGGCTATTGGATTACAGCGAGAT	58	
			Reverse: TAGAATCGGAGGGCGTGAAG		
			Probe: VIC-CAGCGCCGTAACCAG-TAMRA		
	EAV	N	Forward: GGCGACAGCCTACAAGTCACA	204	(4)
			Reverse: CGGCATCTGCAGTGAGTGA		
			Probe: FAM-TTGCGGACCCGCATCTGACCAA-TAMRA		
Conventional PCRs	EHV-1/-4	gB	Forward: CTTGTGAGATCTAACCGCAC	459 (EHV-1)	(13)
	EHV-1		Reverse: GCGTTATAGCTATCACGTCC		
	EHV-4		Reverse: CCTGCATAATGACAGCAGTG	942 (EHV-4)	
	EAV	N	Forward: TCGATGGCGTCAAGA	395	(7)
			Reverse: GGTTCCTGGGTGGCTAATAACTACTTCAAC		

Briefly, 2.0 g of each tissue sample was homogenised with 18 mL of Eagle's minimum essential medium (MEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with a 1% antibiotic solution (Antibiotic Antimycotic Solution; Sigma-Aldrich). Following a low-speed centrifugation, supernatants from tissues from the same foetus were pooled and stored at −80°C until testing.

DNA/RNA extraction and PCR assays. Viral DNA and RNA were extracted from every pool of tissue homogenate according to the NVRI protocol current at the time of submission. For DNA extraction, the procedure included a phenol-chloroform-isoamyl alcohol mixture (28) and the commercial QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Extraction of RNA was performed using TRI Reagent (Sigma-Aldrich) and the QIAamp Viral RNA Mini Kit (Qiagen). Extractions with the commercial kits were performed according to the manufacturer's instructions.

Isolated DNA was tested for the presence of genetic material of EHV-1 and EHV-4 using previously published primers specific to the conserved region of the glycoprotein B gene (9, 13, 27) (Table 1). As a positive control, EHV-1 438/77 (ATCC VR-2229) and EHV-4 405/76 (ATCC VR-2230) reference strains were used, and negative controls (water) were included in each PCR and qPCR run. Each conventional PCR reaction was performed using JumpStart AccuTaq LA DNA Polymerase (Sigma-Aldrich), and the qPCR reactions were performed using TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Warrington, UK).

All of the foetal tissue homogenates were tested for both EHV-1 and EHV-4 using a conventional PCR (1999–2012), while samples received from 2013 onwards were tested using a qPCR. The samples negative for equine herpesvirus 1 and 4 were further tested for the presence of EAV using a conventional reverse transcriptase (RT-PCR) (1999–2012) or a qPCR (2013 onwards).

Ribonucleic acid was tested for the presence of genetic material of EAV by a RT-PCR (from 1999 to 2012) or a quantitative RT-PCR (2013 onwards) with primers and a probe complementary to a highly conserved region within the gene encoding nucleoprotein N of the virus, as previously described (4, 7, 26) (Table 1). As a positive control, the Bucyrus (ATCC VR-796) EAV strain was used, and the negative control consisted of tissue homogenate from an EAV-negative horse. The RT-PCR was performed using a Titan One-Tube RT-PCR Kit (Roche, Mannheim, Germany) and the qPCR was carried out with a QuantiTect Virus Kit (Qiagen).

The changes in sample preparation and PCR tests for EHV-1, -4 and EAV detection implemented in 2012 were associated with improvements to diagnostic procedures introduced in the NVRI laboratory at the time.

Results

No EAV-positive samples were found among the tissue collected from 180 aborted foetuses, also no samples were positive for EHV-4, but 89 (49.4%) were positive for EHV-1 (Table 2). During the first 13 years (1999–2012), a panel of 63 homogenates was tested, of which 20 (31.7%) were EHV-1 positive. During the subsequent 9 years, tissue samples of 117 aborted foetuses were investigated. More than half (n = 69, 59.0%) of the abortions were caused by EHV-1. Overall, a definitive diagnosis was not established in 91 (50.6%) of the cases submitted. In addition, the identification of the origin of 26 of them was not possible, as the complete records were not available.

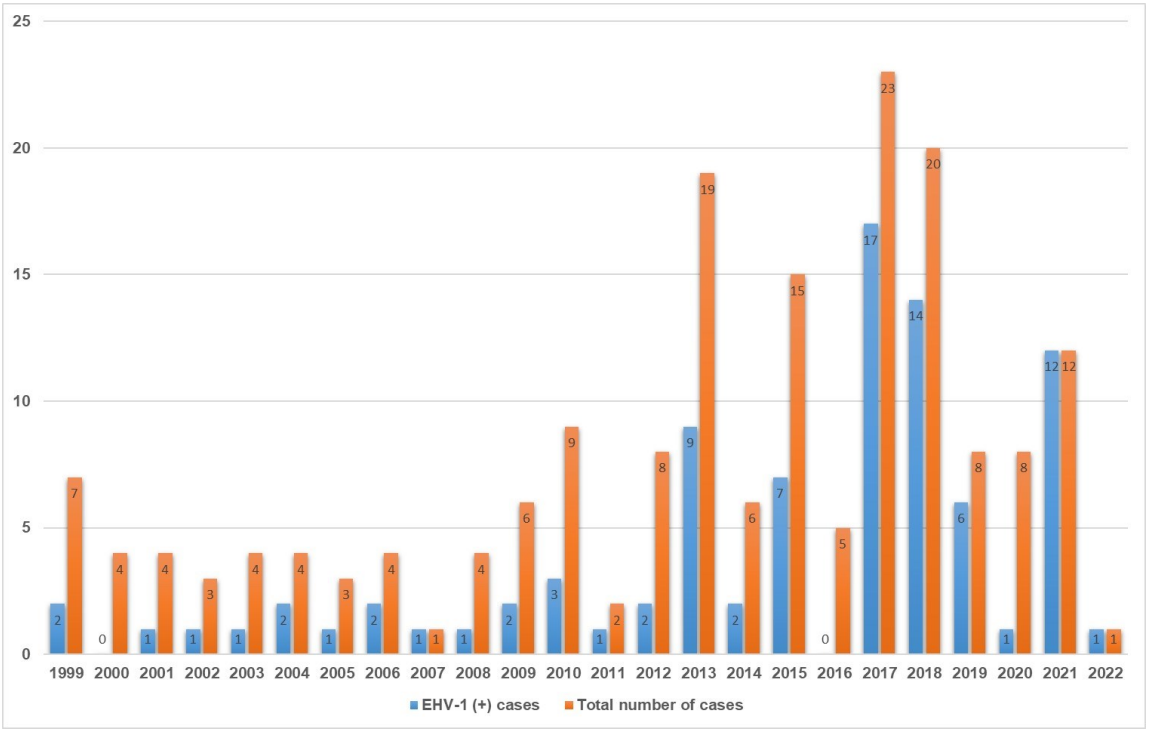

Fig. 2. Numbers of abortion cases caused by EHV-1 in consecutive years between 1999 and 2022

Table 2. The numbers and percentages of abortion associated with EHV-1, -4 and EAV infections between 1999 and 2022

Period[a]	EHV-1		EHV-4		EAV	
	n/N	%	*n*/N	%	*n*/N	%
1999–2012	20/63	31.7	0/63	0.0	0/43	0.0
2013–2022	69/117	59.0	0/117	0.0	0/48	0.0
All	89/180	49.4	0/180	0.0	0/91	0.0

[a] – research periods were distinguished based on the type of PCR used; *n* – number of positive animals; N – all animals tested

The proportion of EHV-1 outbreaks varied for different regions of Poland (Fig. 1). The highest number of abortions due to the virus was identified in the Lubelskie ($n = 34$) and Wielkopolskie ($n = 22$) provinces. A considerably lower prevalence of EHV-1 abortions (n = 1 to 6) was found in the remaining regions.

Between 1999 and 2012, the number of cases submitted for investigation was low and did not exceed nine per year (Fig. 2). During this period, the number of EHV-1–positive cases ranged from 1 to 3 per year, with the exception of the year 2000, when no EHV-1–positive samples were found. In 2013, 2015, 2017 and 2018 the numbers of cases of abortion were significantly higher and reached their peak in 2017. In that year, the highest (n = 17) number of abortions due to EHV-1 was also detected.

Discussion

In the first 14 years of investigation, the number of submitted samples was relatively low. It may be speculated that this may have been due to the limited interest of both field veterinarians and horse owners in the diagnosis of abortions in mares. It could have been caused by financial problems, as during the 1990s many state-owned farms in Poland were transformed into private businesses. Therefore, it would be relevant to analyse samples received from cases of abortion from the earlier years of the period of transformation. Unfortunately, the authors did not have raw EHV-1, -4 or EAV diagnosis data or results from before 1999.

Since 2013, more field veterinarians and managers of large stud farms started to liaise with the Department of Virology at the NVRI, most cases of abortion in mares were reported and appropriate samples were sent for laboratory analysis. Hence, the numbers both of reported abortions and those caused by EHV-1 rose significantly, which was evident in 2013, 2015, 2017, 2018 and 2021. The majority of identified cases of abortion and those of which tissue samples were EHV-1 positive in the present study were from the Lubelskie and Wielkopolskie provinces. This finding may be explained by the presence of numerous stud farms in those regions and increased horse movement. Our study revealed that EHV-1 was responsible for nearly half (n = 89, 49.4%) of the cases of abortion in mares in Poland tested at the NVRI over 23 years. The prevalence of abortions due to the virus differed clearly between published studies. Interestingly, in a recent Polish 34-year retrospective survey, EHV-1 was identified as the cause of 23.5% of abortions (5). A lower prevalence of EHV-1–induced abortions was observed in Michigan (8.9%) (31) and Kentucky (3.3%) in the USA (12), in Canada (10.2%) (21), in the United Kingdom (6.5%) (25), in Normandy in France (6.9%) (15), in Japan (10.1%) (20), in

Denmark (8.0%) (1), in central Italy (21.3%) (19), and in Hungary (16.0%) (30). It is difficult to explain why the proportion of abortions due to EHV-1 was higher than in other countries and in comparison to the recent Polish study. In part, this variation may be explained by differences in the duration of each survey, the local prevalence of pathogens, the number of samples collected, the diagnostic methods used and differences in stud management practices. Other possible explanations might be the irregularity of yearly vaccination programmes and management practice differences between horse studs. Vaccination against equine rhinopneumonitis is one of the key factors in preventing infection in susceptible animals. The duration of immunity following EHV-1 infection or vaccination is short (14). In addition, vaccination does not prevent establishment of EHV-1 infection or latency. Despite these limitations, it is an important part of a herd-health programme, as it reduces the severity of clinical disease as well as the duration and magnitude of nasal shedding (11).

Recent studies confirmed the presence of EAV in Polish horses through the frequent identification of seropositive animals and the detection of viral genetic material in semen samples taken from stallions (26, 29). However, although the virus is known to be an abortogenic agent in horses, in our study none of the analysed cases of abortions seemed to be associated with EAV. Similar results were observed in a previous longitudinal study (1976–2010) on abortogenic viruses in Polish horses, in which the last case of abortion caused by EAV was observed in 2002 (5). This could be the result of increasing awareness of the risk associated with EAV and the introduction of preventive measures in breeding. Equine arteritis virus could be transmitted *via* the respiratory route, venereal route from stallions to mares, or vertically from infected mares to foals. However, a central role in the long-term presence of the virus in the population is played by persistently infected stallions that may shed the virus for many years (3). Therefore, actions directed at the identification, isolation and elimination of such stallions could be effective in limiting the spread of the virus in horses and as a result, the number of abortions induced by EAV in mares. According to European Union regulations, all stallions used for breeding have to undergo serological and virological testing. In Poland, such testing is not universally implemented, but were it to be, it could have a positive effect on limiting the overall risk of EAV infection in mares (33). The administration to mares and stallions of vaccines can be excluded as a factor eliminating abortions caused by EAV. Although several vaccines against EAV have been developed, including the ARVAC1 modified live vaccine widely used in North America and the ARTERVAC1 adjuvanted inactivated vaccine approved for use in some European countries, none of them are available for commercial use in Poland (3, 33). Finally, it is also possible that some of the abortion cases could in fact have been caused by undetected EAV infections. It was previously reported that virus material and lesions caused by infection could not be found in every case in the tissues of aborted foetuses. Instead, abortion may be the result of lesions in the uterus developed during the acute stage of infection (1, 8).

Despite our study only testing for viral agents, similarly to results of recent Japanese and Canadian studies which had the scope to ascribe abortions to causes among a broader range, the causes of around 50% of cases of equine abortions remained unknown (20, 21). In addition, unlike many other investigations, where different methods to determine either infectious or noninfectious causes of abortions were used, the present study has shown that EHV-1 as a viral agent was responsible for nearly half of the cases of abortion in Polish mares. However, this finding should be interpreted with caution, because other unidentified infectious or noninfectious causes may have been present and been parallel causes throughout the whole period of the study.

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