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Original article

A 34-year retrospective study of equine viral abortion in Poland

B.A. Bażanów[1], A.B. Frącka[1], N.A. Jackulak[1], Z.M. Staroniewicz[1], S.M. Ploch[2]

[1] Department of Pathology
[2] IT Lab, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, C.K. Norwida 25, 50-375 Wrocław, Poland

Abstract

The purpose of the present review was a comparison of the abortions caused by EAV and EHV-1 viruses over the 34 years. A total of 452 tissues samples from aborted fetuses (347) or foals (105) stillborn or newborn that died within 72 hours were investigated. The material for the examinations came from different farms located throughout Poland. The tissue homogenates were examined by using virus isolation test in RK-13 and Vero cell lines and the cytopathic agent was confirmed as EHV-1 by the direct fluorescent antibody test or as EAV by the indirect fluorescent antibody test. The study indicated that EAV was isolated (104 cases, 23%) almost as equally often as EHV-1 (116 cases, 25.6%). Both, equid herpesvirus-associated abortion and the abortion induced by EAV were characterized by cyclicity. The percentage of EAV and EHV-1 isolation alternately reduced and increased, but the increase of isolation of one virus was accompanied by the decrease of the other. The domination of one virus over the other occurred in cycles of a few years.

Key words: horse, abortion, EHV-1, EAV

Introduction

Equine arteritis virus (EAV) and equine herpesvirus 1 and 4 (EHV-1 and -4) are considered to be the most common infectious agents that cause abortion in horses all over the world. Both of these viruses pose a serious health threat to horse populations and are distributed worldwide, which leads to economic losses (Timoney and McCollum 1993, Allen 2013). In addition to being responsible for abortion and neonatal death, EAV, EHV-1 and -4 can be associated with respiratory disease (Timoney and McCollum 1993, Crabb and Studdert 1996) and, EHV-1 can also cause neurological disorders (Fritsche and Borchers 2010).

Some of the signs of equine viral abortion caused by EAV, EHV-1 and -4 have clinical similarities, thus definitive diagnosis can only be made with laboratory testing (Timoney 2013). This recognition is crucial because management and prophylaxis are different for each disease. The diagnosis of EHV-1 and -4 is based on traditional methodology such as the isolation of the virus in a cell culture, followed by the seroidentification of the isolated cytopathogenic agent. There are also rapid diagnostic techniques available, for example: ELISA (enzyme-linked immunosorbent assay), PCR (polymerase chain reaction) and many other tests, but generally their use is confined to specialized laboratories (Allen 2013). In contrast, the diagnosis of EAV

Correspondence to: B. Bażanów, e-mail: barbara.bazanow@up.wroc.pl

is based on virus isolation in a cell culture and the identity of a viral isolate should be confirmed by the use of immunoperoxidase staining, immunofluorescence or neutralization tests (Fukunaga and McCollum 1977, Balasuriya and MacLachlan 2007). To confirm infection, detection of nucleic acid by RT-PCR (reverse transcriptase polymerase chain reaction), real time RT-PCR or nested RT-PCR can also be used (Timoney 2013).

The purpose of the present review was the comparison of the abortions caused by EAV and EHV-1 viruses over the 34 years.

Materials and Methods

Tissue samples from 452 aborted fetuses (347) or foals (105) that were stillborn, newborn or had died within 72 hours were collected by the Department of Veterinary Microbiology (Wrocław University of Environmental and Life Sciences, Poland) between 1977-2010.

The specimens received by the laboratory did not include all cases of abortion or death of foals. Abortions caused by non-infectious factors (e.g. twinning, torsion of umbilical cord, progesterone deficiency, congenital defects and other causes) were not included in this study.

The material for the examinations came from different farms located throughout Poland. This research included 10 studs, in which investigations took place over two to fourteen years: 3 farms from Greater Poland Voivodeship (14, 9, 9 aborted fetuses respectively), 2 from Lower Silesian Voivodeship (36, 14), 2 from Silesian Voivodeship (10, 10) and 1 from Lublin (12), Świętokrzyskie (17) and Opole Voivodships (24) and 8 stables in which horses were investigated within one year: 2 farms from Greater Poland Voivodeship (7, 6) and 1 from Lublin (5), Świętokrzyskie (6), Łódź (6), Silesian (6), Opole (26) and West Pomeranian (6) Voivodships. Other aborted fetuses or dead foals came from small holdings situated in different parts of the country (229). In one of the studs (Lower Silesian Voivodeship) abortion monitoring lasted from 1978 to 2002 and during this time 38 aborted fetuses were investigated.

Most fetuses of known age sent to the laboratory were 10 months of gestational age (range from 2 months to term). Unfortunately, in 116 cases the age of the fetuses was unknown. The animals were of various breeds. Abortion and neonatal death took place throughout the year. The liver, lung, and spleen of the aborted fetuses or foals that had died within 3 days after the birth were collected for the analysis.

Tissue homogenates (approximately 10% w/v) were used for virus isolation (VI). Soon after the preparation, the material was inoculated into laboratory glass dishes or 12 or 24-well polystyrene cell dishes that contained RK-13 (rabbit kidney) and Vero (green monkey kidney) cell lines. The inoculated cells were incubated at 37°C. They were examined daily for up to 4 days for the development of viral cytopathic effects (CPE), using an inverted microscope (Olympus Corp., Hamburg, Germany, and Axio Observer, Carl Zeiss MicroImaging GmbH). EHV-1 positive controls were the 22/77 Prudnik and 2973 Golejewko strains (Bażanów et al. 2012) and the Bucyrus strain was used as the EAV positive control. Noninoculated cell cultures were used as the negative control. In the absence of visible CPE, up to 4 subsequent passages were done. The cytopathic agent was confirmed as EHV-1 by the direct fluorescent antibody test or as EAV by the indirect fluorescent antibody test.

Fluorescence isothiocyanate-conjugated anti-EHV-1 (Gamakon, Mevak Slovakia and VMRD Inc., Pullman, USA) was used to confirm EHV-1. To confirm EAV, serum obtained from a seropositive horse immunized by inoculation with Wrocław 2 strain was used, followed by rinsing in PBS and then using fluorescence isothiocyanate-conjugated rabbit anti-horse IgG as the secondary antibody (Sigma catalogue number F7759). Antibodies were inoculated on infected monolayers in glass or plastic laboratory dishes or on coverslips in Leighton tubes, and viewed under an inverted fluorescence microscope (100x and 200x magnification; VEB Carl Zeiss, Jena, Germany, and Axio Observer, Carl Zeiss MicroImaging GmbH, Jena, Germany).

The statistical analysis of the data was performed by using a test for the significance of the Pearson correlation coefficient.

Results

The changes in cell lines characterized as a cellular syncytia (Fig. 1b) were classified as due to EHV-1. If rounding and vacuolization of the cells were observed, and then the cells were detached from the bottom of the laboratory dish (Fig. 1c), the CPE was regarded as caused by EAV.

From 452 aborted fetuses and foals, which were examined between 1977 and 2010, CPE due to the presence of EHV-1 and confirmed by the immunofluorescence test was proved in 116 cases (25.6%), whereas EAV was isolated in 104 (23%). At the beginning of the investigations the percentage of EAV isolation alternately fell and grew. A similar phenomenon was observed in the case of abortion caused by EHV-1, nevertheless the increase of isolation of one

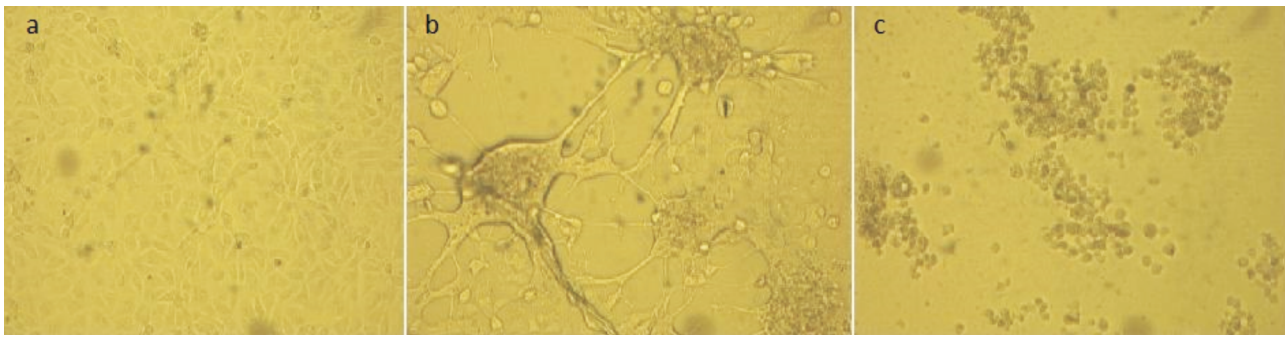

Fig. 1. a – RK-13 cell line, negative control, b – CPE caused by EHV-1, c – CPE caused by EAV. Mag. x100.

Table 1. The quantity and percentage of abortion caused by EHV-1 and EAV between 1977 and 2010.

Year	Cases				
	Total	EHV-1 positive	EHV-1 %	EAV positive	EAV %
1977	10	2	20	8	80
1978	5	3	60	1	20
1979	8	2	25	2	25
1980	2	0	0	0	0
1981	6	0	0	3	50
1982	6	0	0	5	83.3
1983	11	0	0	8	72.7
1984	8	4	50	2	25
1985	21	6	28.5	11	52.3
1986	10	1	10	5	50
1987	5	1	20	1	20
1988	36	7	19.4	5	13.8
1989	65	28	43	14	21.5
1990	20	0	0	10	50
1991	11	0	0	7	63.6
1992	17	1	5.8	0	0
1993	15	15	100	0	0
1994	12	1	8.3	3	25
1995	15	2	13.3	0	0
1996	14	7	50	1	7.1
1997	21	6	28.5	10	47.6
1998	6	0	0	2	33.3
1999	10	4	40	0	0
2000	14	7	50	1	7.1
2001	23	2	8.6	4	17.3
2002	13	3	23.0	1	7.6
2003	11	3	27.2	0	0
2004	4	1	25	0	0
2005	9	3	33.3	0	0
2006	6	1	16.6	0	0
2007	6	0	0	0	0
2008	18	0	0	0	0
2009	5	1	20	0	0
2010	9	5	55.5	0	0

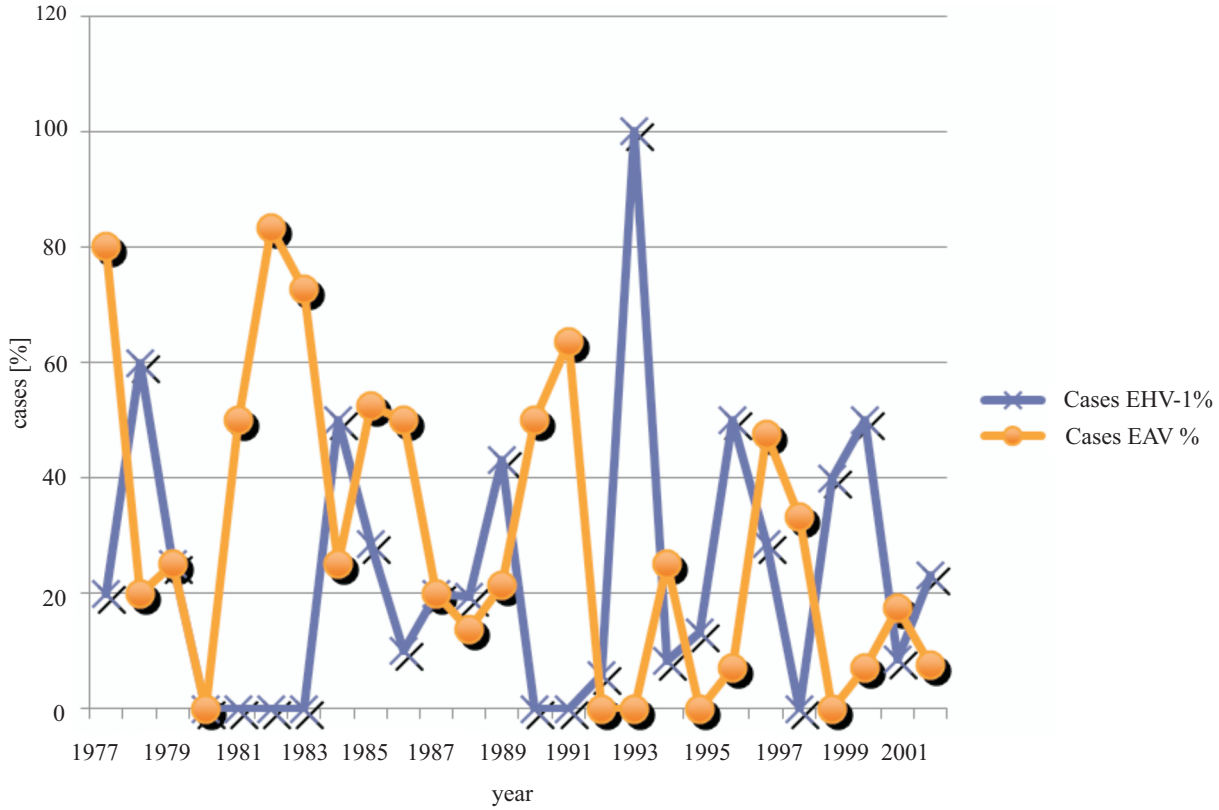

Fig. 2. The percentage of abortions caused by EHV-1and EAV between 1977 and 2001.

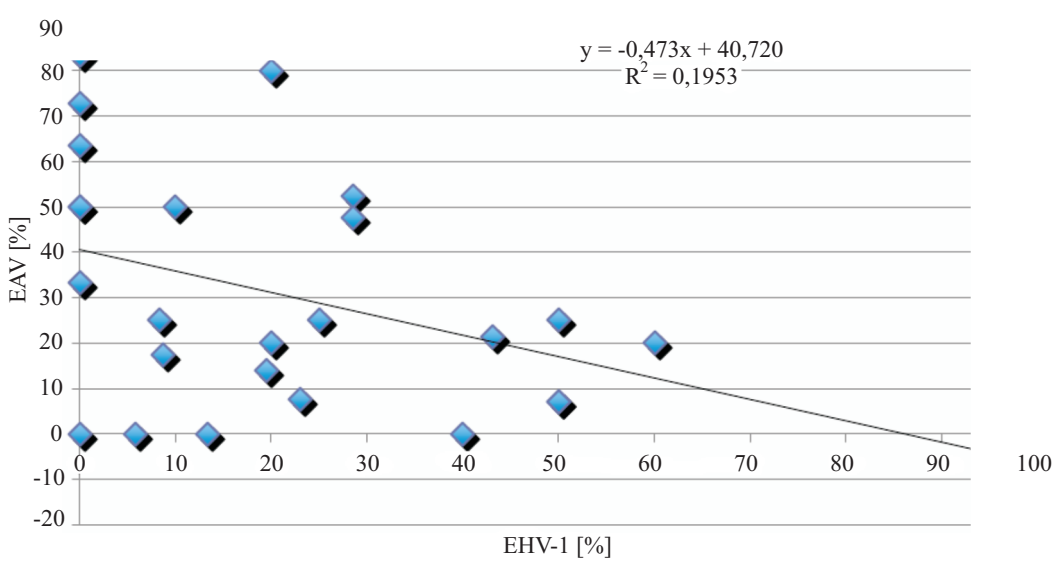

Fig. 3. The correlation between the change of the percentage of abortions caused by EHV-1 and the percentage of abortions caused by EAV.

virus was accompanied by the decrease of the other (Table 1). This situation was observed until 2002. During later years, isolation of EAV has not been conducted in our laboratory, thus only the years 1977-2002 were used for the statistical analysis. The analysis of the data determined the correlation between the percentage ratio of abortions caused by EHV-1 and EAV. Figure 2 illustrates this dependency. Pearson's correlation coefficient for 26-array of two-featured series equals -0.442. This allows us to conclude that there is a weak negative correlation between the variables. In the next step the hypothesis which verifies the statistical significant was tested. The Students t-test was applied with 5% level of signifi-

cance. In result, the hypothesis about the importance of the correlation coefficient was assumed in population (0.05>p>0.02).

The next graph (Fig. 3) shows the reaction of the EAV% (the percentage of abortion caused by EAV) value for the fixed EHV% (the percentage of abortion caused by EHV-1) value. The line plotted on the graph is a simple linear regression – the best-fitting straight line through the points X and Y, which are subsequently represented by the number of abortions triggered by EAV and EHV-1 in a given year. We can observe that it is a decreasing function, because when the percentage of abortion caused by EHV-1 increases, the percentage of abortion caused by EAV decreases.

Discussion

EAV and EHV-1/4 are the causes of equine abortion in approximately 10-50% of cases, depending on the authors and horse populations studied (Tengelsen et al. 1997, Laugier et al. 2011, Bażanów et al. 2012). In general, equine herpesvirus-associated abortions are more prevalent than abortions caused by EAV. Numerous reports from different countries, where the percentage of EHV1 or EHV4 isolation is much higher than the isolation of EAV, confirm this thesis (Szeredi et al. 2008, Laugier et al. 2011). Often, EAV was not isolated at all during the observed period (Giles et al. 1993, Tengelsen et al. 1997, Smith et al. 2003). However, the investigations conducted from 1977 through 2010 in our laboratory indicate that EAV was the cause of abortion in mares almost as equally often as EHV-1. Both equine herpesvirus-associated abortion and the abortion induced by EAV were characterized by the cyclicity. The periods where the rate of isolation of a given virus reached several dozen percent and then declined and again raised appeared alternately. Moreover, in the analyzed period, an interesting regularity was observed: in the time frame when the number of abortions due to EAV grew, the number of EHV-1 isolation from the aborted fetuses fell and vice versa. Unfortunately, although a statistically significant correlation was demonstrated (Fig. 3), too little investigation in particular years and the diversity of places from which the samples came do not allow a unequivocal confirmation of this phenomenon. On the other hand, the 27-year observation within one stud also shows that the abortion caused by EAV and caused by EHV-1 alternated. Notwithstanding, also in this case the correlation is not reliable, because in some years the abortion was not caused by viruses. Another interesting aspect of the analysed data with regard to equine viral abortion in Poland is that the dominance of one virus over the other appeared with a surprising frequency for several years. Perhaps the decrease of the incidence in abortion caused by a given virus among a horse population results from a partial resistance to the virus after the recovery, and the cyclicity of EHV-1 occurrence does not depend on the cyclicity of EAV infection. It is difficult to refer to similar results, because the available papers involve either a shorter observation period (Giles et al. 1993, Hong et al. 1993, Szeredi et al. 2005, Szeredi et al. 2008) or the isolation of EAV occurred only sporadically, e.g. the rate of EAV isolation in France in the years 1986-2009 amounted to 0.005% (Laugier et al. 2011), whereas in Poland, in an comparable period, it was 17.5%. Simliarly in 1988-87 in the United Kingdom it was 0% (Smith et al. 2003) (when it was 22.1% in Poland) and in 1985-89 in the United States (Michigan) it was 0% (Tengelsen et al. 1997) (when it was 26.3% in Poland).

The years 1978-1980 are a notable exception, because the incidence of both infections reduced. In the case of EAV infection this decrease was typical of the general trend, but in the case of EHV-1 infection, it was surprising, particularly since it caused no miscarriages and neonatal foal losses in these years. This could be because during the years 1977-1982 mass EHV-1 vaccination began (Bażanów et al. 2012). A similar situation was observed in Kentucky, where since the implementation of vaccination, EHV has not been as important as other causes of abortion and during 1988 and 1989 foaling seasons only 3.3% of abortions were attributed to the virus (Hong et al. 1993). Unfortunately, in Poland after an initial downturn, the EHV abortion rate increased, probably because the owners did not often adhere to management regulations regarding the use of vaccination and even stopped the vaccination due to the high cost and a low reported efficacy (Bażanów et al. 2012). It is remarkable that the percentage of the abortion caused by EAV was very high when the rate of abortion caused by EHV-1 dropped to zero.

It is difficult to say if the curves of both infections will grow and fall alternately in the future. It seems that this trend may not continue since the isolation of EAV from aborted fetuses in our laboratory has not occurred in the last couple of years. It is unknown if it results from the natural, slow decline of EAV-induced abortion or if the reason is much more mundane: since horse breeding was privatized in Poland in 1990s not all cases of abortion are reported by owners, probably because of the financial cost (Bażanów et al. 2012). This is also reflected in the fact that aborted fetuses and dead foals are sent for investigation to our laboratory less often than previously. On the other hand, al-

though reports concerning the EAV isolation from stallions semen come out relatively often, reports about the abortion caused by EAV appear less frequently than before and the rare outbreaks of abortion are usually the result of the use of the frozen EAV-infected semen (http://aht.org.uk/cms-display/DEFRA_AHT_BEVA_equine_reports.html; http://ca.uky.edu/gluck/q_oct10.asp). Probably the lack of EAV isolation from aborted fetuses during last years in our laboratory is the result of both phenomena.

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