

## SEROPREVALENCE OF MARE HERPES VIRUS IN THE BREEDING SEASON

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*SUMMARY: Equine herpes virus (EHV) is a common virus in populations of equines worldwide, and the two most important viruses in this group are EHV-1 and EHV-4. It is well known that EHV-1 causes respiratory problems, abortion and neurological disease and EHV-4 is typically causing respiratory disease but may lead to abortion. The clinical picture includes: fever - which may be the only symptom, cough, increased amounts of nasal secretions, and without previous, abortions in the late stage of pregnancy and neurological problems that usually involve the last extremity, and urinary tract. These viruses can be detected in a variety of tissues in aborted foals including lung, kidney, liver, spleen and nasal swabs, placenta and blood of live horses. A variety of techniques for diagnostic have been developed which including virus isolation, serology, PCR, real-time PCR and sequencing. The aim of this work was to establish the seroprevalence of herpes virus in reproductive mares. Clinical exams and blood samples were collected from seven mares in one stable which is located in west region of Vojvodina province, the animals are currently used for mating. All the animals in the stable are with a positive finding of antibody titre. The lowest titre of antibodies have animals that have just entered the reproductive cycle. Other symptoms attributed to herpes virus infection was not present, that can be attributed to a latent infection which is present in this case in 100% of the animals in the stable. However, the differential diagnosis should be done by viral isolation from aborted material, because the reproductive problems that occur may be attributed to other viruses.*

**Key words:** herpes virus, seroprevalence, SN test, breeding season, mare.

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### Case report / Prikaz slučaja

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## INTRODUCTION

Equine rhinopneumonitis is the common name for the highly contagious, clinical disease occurs as result of herpes virus infection (OIE Terrestrial Animal Health Code, 2011). Equine herpes virus (EHV) is a common virus in populations of equines worldwide, and the two most important viruses in this group are EHV-1 and EHV-4 (Lohmann, 2008). It is well known that EHV-1 causes respiratory problems, abortion and neurological disease and EHV-4 is typically causing respiratory disease but may lead to abortion.

Equine herpesvirus-1 (EHV-1) was first described by Drs. W.W. Dimock and P.R. Edwards at the Kentucky Agriculture Experimental Station, Lexington in the early 1930s (Kydd et al., 2006). Equine herpesvirus-1 and 4 are DNA viruses belonging to subfamily Alphaherpesvirinae (Patel and Heldens, 2005), genus Varicellovirus, and in fact, the two viruses were considered as subtypes of one virus species until the early 1980s (Bresgen et al., 2012).

However, in the 21st century, over 70 years after its first description and despite many vaccine trials, the virus remains responsible for major economic and welfare problems, causing respiratory and neurological disease and abortion in pregnant mares (Kydd et al., 2006). Within the United Kingdom, approximately 6% of equine abortions are caused by EHV-1, with occasional further cases linked to the closely related virus EHV-4 (Smith et al., 2003).

The virus can be transmitted from an infected animal through nasopharyngeal secretions, through the fetus, fetal membranes and reproductive tract or by reactivation of the virus from the latent state (Allen, 2002). Other authors indicate that both viruses enter via the respiratory tract where a first lytic replication takes place in the respiratory epithelium (Bresgen et al., 2012). After primary replication in the nasal mucosa, EHV-1 but not EHV-4 is capable of efficient infection of leukocytes present in the tributary lymphoid tissue, which then results in a leukocyte-associated viremia. From there, EHV-1 is spread and can reach the end vessels of the pregnant uterus or the central nervous system (CNS), particularly those of the spinal cord (Edington et al., 1986; Patel and Heldens, 2005). In the case of abortion, virus can transgress layers of the maternal and fetal placenta and result in infection of the fetus and subsequent abortion, although “sterile” abortions following endothelial cell infection are also observed (Smith and Borchers, 2001; Smith et al., 1996). Viruses are infectious for all equines but donkeys are often latently infected. The virus can not be transmitted to humans. The incubation period is short from the 24 hours up to 4-6 days, but if it comes to abortion the incubation period may take several months from infection. EHV-1 and EHV-4 infections become latent involving lymphoid tissue and the sensory and trigeminal ganglions (Allen, 2006; Pusterla et al., 2010).

The most devastating manifestations of this viral infection is a mass phenomenon of abortion, and perinatal mortality with mioencefalopathy (Dixon et al. 1978; Hartley and Dixon 1979; Greenwood and Simpson 1980, Mumford et al., 1987; McCartan et al. 1995). The clinical picture includes: fever - which may be the only symptom, cough, increased amounts of nasal secretions, without previous abortions and other characters in the late stage of pregnancy, neurological problems usually involve the last extremity, and urinary tract (ataxia, urinary retention or incontinence of urine and atony of the bladder) with preserved consciousness (Lohmann, 2008).

These viruses can be detected in a variety of tissues in aborted foals including lung, kidney, liver, spleen and nasal swabs, placenta and blood of live horses. A variety of techniques have been used to detect and differentiate EHV-1 and EHV-4 in horses including virus isolation, serology, PCR, real-time PCR and sequencing (Varrasso et al., 2001; Pusterla et al., 2005; Diallo et al., 2007; Marenzoni et al., 2008; Smith et al., 2010). OIE recommend for serological diagnosis to use virus neutralizing test, ELISA or CFT (OIE Terrestrial Manual, 2008.).

Equine herpes virus can become latent in around 80% of horses (Welch et al., 1992), involving lymphocytes and the trigeminal ganglion (Borchers et al., 1999). The prevalence in aborted fetuses vary in different studies: in Turkey the prevalence was 42% (Turan et al., 2012), in France about 15% (Leon et al., 2008), in Kentucky 54% (Allen et al., 2008), in the UK 7% (Smith et al., 2003) and in the USA 4% (Giles et al., 1993).

It is suggested that transfer of maternal VN antibody could not prevent infection, at least of the upper respiratory tract, but did provide clinical protection (Kydd et al., 2006). Virus positive nasal swabs detected as early as 11 days post partum (Foote et al., 2004).

There are attenuated and inactivated vaccines, but the need for greater protection for repeated vaccinations given program depending on the manufacturer. There is quite a body of literature and as much debate as to which vaccines and vaccine regimens provide the most reliable protection against EHV-1- and EHV-4-induced disease. But only vaccine with a claim against EHV-1-induced abortion is an inactivated EHV-1/EHV-4 combination vaccine (Heldens et al., 2001)

## MATERIALS AND METHODS

Clinical exams and blood samples were collected from seven mares in one stable which is located in west region of Vojvodina province. All mares are kept loose in a common outlet during the day and at night in individual pens. The animals are currently used for mating, but in the past they were used for shows and racing. There were no action in any form of competition for more than two years for each of the mares. On all animals were conducted a thural clinical exam with emphasis on respiratory and neurological disorders. Along with taking the blood samples and clinical exams there was conducted anamnestic questioning of the owner and trainers of these animals and with an emphasis on reproductive, respiratory and neurological disorders.

Blood samples were taken from v. jugular with all the principles of asepsis and antisepsis and serological analysis was performed by serum neutralization test as directed from OIE, Chapter 2.5.9. Terrestrial Manual 2008. In this test positive are those animals who have antibody titre 1:2 or greater.

## RESULTS

Table 1 shows the results of serological tests and medical history. According to the owner all mares, except the two jangest, had trouble with the conception. All animals has no other clinical findins then reproductive.

Table 1. Research results shows that 5 out of 7 animals are with reproductive issues and that all animals have positive finding of antibody titre

No.	Breed	Age (year)	Origin	Vak c.	Clinical findings	No. of foals	Ab titre
1	Lipizzaner	7	Domestic	No	-	The first mating	1:16*
2	American trotter	6	Domestic	No	-	The first mating	1:32*
3	American trotter	13	Domestic	No	Death of newborn foals, poor conception	1	1:32*
4	Lipizzaner	12	Domestic	No	Death of newborn foals, poor conception, abortion (twins)	2	1:64*
5	American trotter	20	Domestic	No	Death of newborn foals	8	1:128*
6	American trotter	9	Domestic	No	Poor conception	1	1:256*
7	American trotter	14	Import	No	Death of newborn foals (2x)	3	1:256*

\*All mares have titre higher than 1:2 which is considered positive.

## DISCUSSION

The table shows that all the animals in the stable are with a positive finding of antibody titre which is greater than 1:2. The lowest titre of antibodies have animals that have just entered the reproductive cycle. Older animals and those that have had multiple mating shows a higher antibody titres. The animals had reproductive problems, except the youngest two mares that had just one mating. The aforementioned reproductive problems - abortion and newborn foal deaths are consistent with the findings of many authors who state that the most devastating manifestations of this viral infection is a mass phenomenon of abortion, and perinatal mortality with mioencefalopathy (Dixon et al., 1978; Hartley and Dixon, 1979; Greenwood and Simpson, 1980; Mumford et al., 1987; McCartan et al., 1995). Other symptoms attributed to herpes virus infection was not present, that can be attributed to a latent infection which is present in this case in 100% of the animals in the stable. This is consistent with findings of other authors which find that the latent infection rate in horses is about 80% or higher (Welch et al., 1992). It is also important to mention that there was no vaccination program for this animals which may help to reduce the consequences of infection. Abortion, death of newborn foals and poor conception can be attributed to other causes, and unfortunately in this case there was no available the aborted material for definitive confirmation of diagnosis and virus isolation.

## CONCLUSION

From the obtained results it can be concluded that in our herds there are a great number of latently infected animals. The predominant clinical forms of infection are reproductive problems, which include abortion, death of newborn foals and a bad conception. However, the differential diagnosis should be done by viral isolation from aborted material, because the reproductive problems that occur may be attributed to other viruses. Vaccination program is essential for managing of this infection.

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## SEROPREVALENCA KONJSKOG HERPES VIRUSA KOD KOBILA U SEZONI PARENJA

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### Izvod

Konjski herpes virus (EHV) je uobičajeni virus u populacijama kopitara širom sveta, a dva najvažnija virusa u ovoj grupi su EHV-1 i EHV-4. Dobro je poznato da EHV-1 uzrokuje respiratorne probleme, abortuse i neurološke probleme a da EHV-4 obično izaziva respiratorni oblik bolesti mada može dovesti i do abortusa. Klinička slika obuhvata: povišenju temperature - što može biti jedini simptom, kašalj, povećanje količine nosnog sekreta, i bez prethodnih znakova može doći do abortusa u kasnoj fazi graviditeta. Neurološki problemi obično obuhvataju zadnje ekstremitete i urinarni trakt. Ovi virusi mogu biti detektovani u različitim tkivima abortiranih ždrebadi, uključujući pluća, bubrege, jetru, slezinu i briseve iz nosa, placenti i krvi živih konja. Raznovrsne tehnika za dijagnostiku su razvijene koje uključuju izolaciju virusa, serologiju, PCR, RT-PCR i sekvencioniranje. Cilj ovog rada je bio da se utvrdi seroprevalenca herpes virusa kod kobila u reprodukciji. Klinički pregledi i uzorci krvi su prikupljeni od sedam kobila u jednoj štali koja se nalazi u zapadnom delu pokrajine Vojvodine, životinje se trenutno koriste za parenje. Sve životinje u štali su sa pozitivnim nalazom antitela. Najniži titar antitela imaju životinje koje su upravo ušle u reproduktivni ciklus. Ostali simptomi pripisani herpes virusima nisu bili prisutni, što se može pripisati latentnoj infekciji koja je prisutna u ovom slučaju kod 100% životinja u štali. Međutim, diferencijalnu dijagnozu treba uraditi uz pomoć izolacije virusa iz abortiranog materijala, jer se reproduktivni problemi koji se javljaju mogu pripisati i drugim virusima.

**Ključne reči:** herpes virus, seroprevalenca, SN test, sezona parenja, kobila.

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