VETERINARSKI ARHIV 81 (2), 175-186, 2011

Control of equine arteritis virus (EAV) on stud farm

Peter Hostnik[1]*, Sara Mankoč[1], Ivan Toplak[1], Igor Klobučar[2], Tadej Malovrh[1], and Jože Grom[1]

[1]*Institute of Microbiology and Parasitology, Virology Unit, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia*

[2]*Clinics for Reproduction and Horses, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia*

HOSTNIK, P., S. MANKOČ, I. TOPLAK, I. KLOBUČAR, T. MALOVRH, J. GROM: Control of equine arteritis virus (EAV) on stud farm. Vet. arhiv 81, 175-186, 2011.

ABSTRACT

An epidemiology of infection with equine arteritis virus (EAV) on one stud farm with approximately 350 horses in the period from 1995 to 2008 was studied. Infection was detected by virological methods, using a virus neutralisation test (VNT) for EAV antibody detection in serum samples, and virus isolation and RT-PCR test for virus detection in semen. No clinical picture of the disease was observed. The highest seroprevalence (nearly 100%) was among stallions and old mares, while seroprevalence among young fillies, before mating, was lower than 9%. A high incidence for seroconversion was detected among fillies after mating. Virus was detected by RT-PCR and by a virus isolation test in the semen of 40.7% of 76 seropositive stallions. The 8 stallions, which were shedding EAV, were infected within the period of the first three years after birth, but the other 12 seropositive stallions, which were negative for EAV in semen samples, became firstly seropositive 5 years after birth. In this study we confirmed that the major transmission of EAV on the stud farm occurred from shedding stallions to fillies during the mating time, but an important role of virus transmission to other horses is also played by contact between different groups of animals. Virus positive stallions were castrated and a new breeding unit for young foals was established. EAV negative foals were vaccinated and were bred outside the farm up to 3 years of age.

Key words: equine arteritis virus, transmission, diagnosis, horse, eradication

Introduction

Equine viral arteritis (EVA) is a contagious, primarily a respiratory disease of equids that may manifest as pyrexia with mild respiratory signs, limb and ventral oedema, rhinitis, conjunctivitis, as well as abortion in pregnant mares, pneumonia in young horses and mortality in neonates. The causation agent do not usually kill adult horses, mortality

*Corresponding author:

Prof. Dr. Peter Hostnik, PhD., Institute of Microbiology and Parasitology, Virology Unit, University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia, Phone: +386 40 398 310; Fax: +386 1 4779 352; E-mail: peter.hostnik@vf.uni-lj.si

ISSN 0372-5480
Printed in Croatia

is evident among young foals and abortions in pregnant mares. Abortion rates in EVA infected mares vary from 10 percent to 70 percent. Subclinical infections are more common than clinical disease (TIMONEY and McCOLLUM, 1993).

Equine arteritis virus (EAV) was firstly isolated from foetal lung, collected during an epizootic of abortion in Bucyrus, USA (BRYANS et al., 1957). The disease is caused by the equine arteritis virus, classified as a member of the order *Nidovirales*, within the family *Arteriviridae*. There is only one serotype of EAV that has been recognised using convalescent equine sera in a cross neutralisation test, although pathogenic, antigenic and genetic differences exist between the viral isolates (GLASER et al., 1995). Humoral immune response is directed against two major viral antigens, large envelope glycoprotein (GL) and (N) proteins. GL protein induces neutralizing antibodies in horses and has been widely studied for use in diagnostic tests and vaccines (CHIRNSIDE et al., 1995).

During an outbreak, infected horses shed the virus in secretions and excretions for a limited period via the respiratory tract. The virus is transmitted venerally by natural or artificial insemination of mares from carrier stallions or by contact with aerosol during acute respiratory infection (TIMONEY et al., 1987; TIMONEY and McCOLLUM, 2000). Virus can also be transmitted vertically through congenital infection when mares are infected in late gestation (GLASER et al., 1997). After infection, stallions may become asymptomatic, long term carriers of the EAV and can disseminate the virus to susceptible mares. Several findings suggest that persistence in the accessory sex glands of stallions is testosterone dependent (TIMONEY et al., 1987).

Infected horses develop a specific antibodies which can be detected in blood samples for several years. Mares naturally eliminate the virus after acute infection and develop strong immunity to re-infection. Newborn foals aged between 2 and 6 months are resistant to virus infection, as a result of colostral protection given by their seropositive mothers (McCOLLUM, 1976). Stallions also develop specific antibodies, but 30 do 60% of seropositive stallions are persistently infected. Carrier stallions, which shed EVA, are always seropositive. After an acute infection horses shed the virus for a limited time or permanently in their semen, because EVA can replicate in the ampulles of the vas deferens of the accessory organs. The percentage of horses which are positive for antibodies may differ between countries. Many studies have been shown that the percent of seropositive horses has increased in Germany, Sweden and Italy (GLASER et al., 1995; HUNTINGON et al., 1990).

A virus neutralisation test is used as a standard test for serologic detection of EAV antibodies (CHIRNSIDE et al., 1995). Enzyme-linked immunosorbent assay (ELISA) is another rapid method for screening a large number of samples. The presence of antibodies may not indicate an active infection (DUTHIE et al., 2008). The presence of EAV in clinical samples or semen samples can be identified by virus isolation in cell culture (TIMONEY

et al., 1986) or by polymerase chain reaction - PCR (GILBERT et al., 1997). It is crucial to identify the persistent infection of carrier stallions due to the fact that these animals represent a natural virus reservoir. The most suitable method for discovering the EAV in semen samples of carrier stallions is RT - PCR and could successfully replace virus isolation in cell culture tests (ST-LAURENT et al., 1994).

In order to minimize the spread of infection and economic losses, control programmes, including prophylactic vaccination programmes, have been implemented in several countries. Some countries have imposed controls on the movements of carrier stallions and EAV infected semen. The import of carrier stallions and infected semen into some countries is not allowed (HORNER, 2004). In Slovenia, all seropositive pedigree stallions are controlled for viruses in their semen and they must be negative. Control of the disease in stallions is notified, but control of respiratory outbreaks is voluntary.

This paper describes the current serological and virological status of horses on a large stud farm and can be used as a guideline for control and eradication of the disease.

Materials and methods

Groups of animals on a stud farm in year 2002. The research environment is a stud farm, with approximately 350 horses (including 142 mares), which is located outside the urban region. Groups of horses are situated in three separated buildings and all movements between the groups are strictly controlled. All pedigree stallions are in stable No 1. Horses older than three years (the owner's mares and geldings), which are used for riding lessons, are located in stable No 2. The building for mares and foals (stable No 3) is divided into parts with a stall to which the pregnant mares are moved just before foaling, a stall for pregnant mares and fillies, a stall for mares with foals until weaning and a stall for 1-3 year old fillies and barren mares. There is a large paddock between the stables into which the colts and fillies are released when the weather is unsuitable for grazing. Since 2003, all colts, aged between 1 and 3 have been moved into stable No 4, located 3 km away from the stud farm.

Serological testing. The serological status of the horses on the stud farm has been monitored each year since 1995. In 1995 (372 samples), 2002 (309 samples) and in 2006 (359 samples) all the horses on the farm were tested by a serum neutralisation test, in the other years only previous seronegative horses and stallions were included in the study. The EAV control program on the stud farm has been carried out since 2002. Starting in 2002, all serologically negative colts aged more than 10 months were first removed to quarantine, where the second serological examination was conducted. All colts with a second negative result were removed from quarantine to a separated holder (stable No 4). Colts born in 2003, 2004 and 2005 were bred in stable No 4, where they were regularly tested for antibodies against EAV. In 2006 all negative colts were vaccinated

with the attenuated vaccine Artevac® (Ft. Dodge, Animal Health, USA) following the manufacturer's instructions. After the second dose of vaccine, the vaccinated colts were moved back from stable No 4 to stable No 3. Mares were not vaccinated and seronegative mares were tested for antibodies against EAV every year. They were used as indicators of virus transmission on the stud farm.

The serological status of horses on the farm has been monitored through regular serological screening since 1995. Blood samples have been collected from horses and serological tests performed. A vaccination program was started in 2006. At that time, 359 animals were on the farm. Serum samples were collected, frozen and stored at -20 °C until analysis. All samples were tested by a virus neutralization test (VNT) for detection of specific antibodies according to the O.I.E. manual. Serial two-fold dilutions of each serum were mixed with 100 $TCID_{50}$ of EAV, Bucyrus strain (ATCC VR-796) in 96-well microtitre plates, using two wells for each serum-virus mixture. 10% guinea pig serum was added as a complement to the medium, Advanced D-MEM, InvitrogenTM (Gibco®, Scotland, UK). The plates were incubated for 60 minutes at 37 °C and then about 200,000 RK13 (RK-13, ATCC CCLR7) cells in a volume of 0.1 mL were added to each well. Plates were incubated at 37 °C in 5% CO_2 atmosphere. Readings were made on the 3rd day, where CPE in the virus control culture was completed. The titre of each serum was reported as the reciprocal of the cell monolayer against challenge exposure, with approximately 100 tissue-culture infectious doses of the EAV-Bucyrus strain. Horses with a titre higher than 1:4 are considered to be positive.

Collection of semen samples from stallions. In each case of an EAV serological positive stallion, semen was collected from individual stallions, and the virus was detected by virus isolation and RT-PCR tests. Semen was collected from 76 stallions using an artificial vagina. Two semen samples were collected in a yearly period and 2 to 14 days elapsed between the collections. The sperm rich fraction of the ejaculate was mixed in a ratio of 1:5 with a minimal essential medium (MEM - CMC, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) containing the antibiotic and immediately sent to the laboratory. The sol phase and sperm rich fraction was separated by centrifugation and supernatant was divided into 1.5 mL tubes and stored at -70 °C or used immediately. Sperm from all pedigree stallions and also from other stallions was collected for virus isolation and RT-PCR. Virus positive stallions were castrated or eliminated. During the control period between 2003 and 2007, a total of 240 semen samples were collected from 76 seropositive stallions and were tested for the presence of EAV.

Virus isolation. A rabbit kidney (RK-13) cell line was maintained in advanced D-MEM with 5% foetal bovine serum and antibiotics (Antibiotic/antimycotic, Gibco). Cells were grown in a humidified atmosphere containing 5% CO_2 at 37 °C. The supernatant of semen samples was diluted 1:10, 1:100 and 1:1000 and 0.5 mL of each dilution was

inoculated on a confluent monolayer of RK-13 growing in six well microplates. After incubation at 37 °C for 1 hour, the monolayer was washed with MEM and replaced with the growing medium (MEM, Invitrogen™, Gibco®, Strathclyde, UK), supplemented with 2% bovine foetal serum containing 0.75% (w/v) carboxymethyl cellulose (CMC, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Cultures with samples were observed daily for the cytopathic effect (CPE). Samples without CPE on the 7th day were frozen and their passage into fresh cells was performed. Isolates of EAV were confirmed in an indirect peroxidase test using specific monoclonal antibodies (Bio-x Diagnostics SPRL, Jemelle, Belgium).

RT-PCR test. Viral RNA was isolated from the supernatant obtained by centrifugation of the sperm samples. The ORF 7 (nucleocapsid gene) was amplified using the access one step RT-PCR kit (Promega, Madison USA) as previously described (MANKOČ et al., 2007).

Results

During this period the horses were observed by clinical inspection for symptoms of the disease as described by different authors. The clinical signs of the disease, such as oedema of the limbs, conjunctivitis, palpebral oedema and abortions, were not noticed.

Table 1. Serological status category of horses on the stud farm in 1995 and 2002

Year	Category of horses	No. of tested horses	No. of antibody positive	% of positive animals in category
1995	Foals	42	0	0.0
	Mares with foals	42	30	71.4
	Young fillies before mating	69	4	5.8
	Colts (aged between 1 and 3)	37	18	48.6
	Mares without foals	31	25	80.5
	Riding horses	79	41	51.9
	Pedigree stallions	72	59	81.2
	Total	372	177	47.5
2002	Foals	38	12	31.6%
	Mares with foals	40	38	95.0%
	Young fillies before mating	70	6	8.5%
	Colts (aged between 1 and 3)	33	32	96.9%
	Mares without foals	12	11	91.6%
	Riding horses	57	45	78.9%
	Pedigree stallions	59	59	100.0%
	Total	309	217	70.2%

The results of the seroneutralisation test detecting antibodies against EAV in horses (in year 1995 and 2002) on the stud farm is presented in Table 1. In 2002 only 12 foals (31.6%) out of 38 were seropositive. All positive foals were younger than 6 months and almost all their mothers were also found seropositive. In the group of 70 young fillies, before mating, only 6 were found seropositive. In the group of 12 mares, which had not become pregnant and were without foals, 11 of them were seropositive. Very high seroprevalence was found among colts (96.9%), aged between one and three years, among pedigree stallions (100%) and riding horses (78.9%). The seroprevalence increased from 47.5% in 1995 to 70.2% in 2002.

Serological results (Table 2) after implementing control measures showed that all colts younger than 3 years and fillies younger than 4 years were negative for antibodies against EAV. Young fillies became seropositive when they were exposed to infected stallions. Sero-prevalence in the male group, older than three years, was 100%.

Table 2. Results of antibodies against EAV in 2006 according to sex and age (all results were obtained prior to vaccination, which started in 2006)

Age (years)	Sex (Female)	No. of tested	No. of positive	Percent	Sex (Male)	No. of tested	No. of positive	Percent
1	F	19	0	0	M	23	0	0
2	F	23	0	0	M	14	0	0
3	F	20	0	0	M	15	0	0
4	F	15	0	0	M	8	8	100
5	F	17	3	17.6	M	18	18	100
6	F	16	1	6.2	M	6	6	100
7	F	10	6	60.0	M	11	11	100
8	F	11	7	63.6	M	9	9	100
9	F	6	5	83.3	M	10	10	100
10	F	6	6	100	M	13	13	100
>10	F	41	40	97.5	M	48	48	100
Total		184	68	36.9		175	123	70.3

The virus was detected in the semen of 31 stallions (in 40.7% of seropositive stallions). EAV positive stallions were excluded from the reproduction process, eliminated from the stud farm or castrated.

The results of virus detection in twenty stallions which became seropositive during the observation period are presented in Table 3. The semen of these stallions was tested at least twice for virus isolation and RT-PCR. EAV was never detected in the semen of 12 stallions by either of these methods. The EAV was detected in more than one semen sample of 8 stallions. The stallions, which became persistently infected, were infected

with EAV within the period of three years after birth, but other 12 seropositive stallions, which were EAV negative in semen samples, became seropositive after 5 years from birth. None of the stallions in this study infected as adults became carriers.

Table 3. Results of virus isolation and RT-PCR tests compared to the results of VNT and year of first VNT positive result among 20 stallions

		Detection of EAV antibodies		Detection of EAV	
Number of stallion	Year of birth	First infection at the age	Results of VNT (with titre) in year 2007	RT-PCR	Virus isolation
001	1989	14	Pos. (1:64)	Neg.	Neg.
103	1993	10	Pos. (1:4)	Neg.	Neg.
164	1995	8	Pos. (1:128)	Neg.	Neg.
225	1998	5	Pos. (1:128)	Neg.	Neg.
331	2000	6	Pos. (1:1024)	Neg.	Neg.
353	2001	5	Pos. (1:64)	Neg.	Neg.
363	2001	5	Pos. (1:32)	Neg.	Neg.
838	1983	15	Pos. (1:32)	Neg.	Neg.
857	1984	13	Pos. (1:32)	Neg.	Neg.
919	1986	10	Pos. (1:16)	Neg.	Neg.
973	1988	9	Pos. (1:64)	Neg.	Neg.
859	1984	19	Pos. (1:32)	Neg.	Neg.
042	1993	3	Pos. (1:512)	Pos.	Pos.
085	1993	3	Pos. (1:512)	Pos.	Pos.
150	1995	2	Pos. (1:128)	Pos.	Pos.
168	1996	3	Pos. (1:32)	Pos.	Pos.
213	1997	2	Pos. (1:2048)	Pos.	Pos.
344	2002	3	Pos. (1:512)	Pos.	Pos.
840	1993	2	Pos. (1:128)	Pos.	Pos.
926	1993	2	Pos. (1:64)	Pos.	Pos.

Discussion

In our study we tried to implement a control programme and reduce the incidence of EAV transmission on a large stud farm. We used the method of grouping animals into categories to prevent virus transmission and monitoring of antibodies in serum and EAV in semen.

On this stud farm, the infection of horses with EAV was serologically diagnosed for the first time in 1995. At that time 47.5% seroprevalence was detected (Table 2). Seroprevalence has increased from year to year. We can conclude that EAV has circulated

between the animals, because seronegative foals become infected continuously. Vaccination against EAV was not practiced at that time. In 2002 the seroprevalence on the stud-farm reached the level of 70.2%. Results of all monitoring showed that the highest prevalence in groups of stallions (100% in 2002), riding horses older than three years (51.9% - 78.2%) and mares with foals (71.4% - 93.5%) was detected. Seroprevalence among foals aged between 6 to 12 months was very low (from 0 to 9.3%) and also the group of young fillies before mating always had seroprevalence lower than 10% confirmed.

In the summer of 1995 EAV infection was recorded for the first time on the stud farm. The diagnosis of EAV was confirmed by serological testing. There is no data when the infection occurred on the stud farm first time. Vaccine against EAV was not available before 2006, thus all specific antibodies to EVA were detected as natural infection. Horses on the farm did not show clinical signs of the disease and the abortion rate was lower than 2%. SZEREDI et al. (2005) reported sporadic abortions in EAV infected herds. The disease often remains clinically undetected, because some strains of EAV are considered to be low virulent (MITTELHOLZER et al., 2006). Results of VNT between 2002 and 2006 showed that more than 70% of horses were seropositive to EAV. The seroprevalence in the female group was lower, 36.9%, while in the group of male horses the seroprevalence was much higher, 70.3% (Table 2), which is comparable to the incidence of seropositive animals on infected farms reported by others (HUNTINGTON et al., 1990). The fact is, that the percentage of infected horses increased with age, but mares became serologically positive at the age of 4 to 5, most of them after mating. This may be an indicator that virus transmission was usually the result of veneral contact, because young fillies were always negative and mares became seropositive after weaning. The low prevalence in fillies before mating indicates that lateral aerosol spread may be a minor route of infection overall. It is interesting that young fillies did not become infected although they were together with seropositive adult mares in the grazing paddock during the summer period. During that time (during the grazing season) active infection among this group of horses may not be present. If mares or fillies were infected at mating in the winter period, according to the literature data, mares become shedders of EAV soon after infection, so they cannot infect seronegative fillies during the summer time when they are in close contact. According to the rules of the technology breeding on a stud farm the female horses maintained for reproduction are divided into four groups. These four groups are only in contact during the summer period in the paddock, at other times they are separated and one group does not have direct contact with the animals from the other group. During the mating period, pregnant mares are together with their foals, and are situated separately from other horses. In the case of infection during the weaning period and during incubation and the viremia period they do not have contact with young fillies, but later during the summer period of the year when they are in close contact obviously they no longer secret the virus. Our result suggests that veneral transmission by the EAV positive stallions is the major route

of infection. Indirect spread of the virus by aerosol may be a minor route of infection, because young fillies maintain their negative status until mating although they live close to the infected mares.

Colts became serologically positive at the age of 1 to 2 years, most of them when they come into contact with stallions and racehorses. EAV can spread directly by contact to infect other stallions on the property (GUTHRIE et al., 2003). With the results shown in Table 1 and Table 2, we can confirm that the preventive measure of breeding colts in a separate location, where they have no contact with other horses, was successful and all colts remained seronegative over a three year period. According to the results of serological monitoring from 1995 to 2002 and knowledge of breeding technology, it has been concluded that most colts became infected after transfer from the mother to a colts' group in the period from 1-2 years of age when they were in contact with older colts. Lateral transmission of EAV from shedding stallions in contact with susceptible horses is well known (GUTHRIE et al., 2003). The isolation of seronegative colts in stable No 4 and separate location for breeding successfully protected them from EAV infection. The stud farm breeding facilities permit separate breeding of colts until the age of three years and after that period of time they should come back to the stud farm. As there was no other long term possibility to protect negative colts against EAV infection, an annual vaccination program of all seronegative non-carrier stallions and colts was adopted. No vaccine virus strain has ever been isolated from semen after vaccination. Virus neutralisation antibodies to the vaccine strain persist for at least 2 years (TIMONEY and McCOLLUM, 1988). The protective titre of the antibody is 1:43 and was determined with VNT (FUKUNAGA et al., 1991).

A virus isolation test in cell culture and RT-PCR assay were used to detect individual horses which are carriers, shedding EAV. The EAV strain isolated on this stud farm produced barely visible CPE on the RK-13 cell culture and in the case of virus isolation, only a few cells were positive in the immunoperoxidase test, so the RT-PCR test was very useful. By the parallel testing of samples by RT-PCR and virus isolation on cell culture, we could prevent a false negative diagnosis. Isolates of EAV from the stud farm were genotyped in ORF1b and the results of phylogenetic studies showed 97.1 - 98.3% identity at the nucleotide level with the most closely related strain, Wiena (MANKOČ et al., 2007).

By serological monitoring we examined the correlation between the seropositive carriers and seropositive non-carriers of EAV and the first time of infection with EAV. The seropositive stallions in this study were horses from one to nineteen years old. The presence of EAV in semen samples was detected in 8 seropositive stallions, all infected in the period before three years after birth. EAV was not detected in semen samples of the other 12 seropositive stallions infected after five years after birth. Our results suggest that

stallions become carriers if they are infected during the period of the first three years of life. The most important key point for successful control of EAV is the diagnostic method for virus detection in stallion sperm and identification of virus shedders (HUNTINGON et al., 1990).

Our goal for implementing the control programme on the stud farm is to produce non carrier stallions. According to the analysis of serological and virological results and other factors, it was decided to start a vaccination program on that stud farm. All seronegative stallions and colts were vaccinated at the end of 2005. A modified live vaccine called Artevac was used.

Although this paper reports the detection of EAV infection in a micro location, the transmission model of spreading EAV in a horse population gives us some important information about controlling the disease and how we can produce non-carrier stallions in infected populations. In conclusion we can confirm, that a less virulent form of EAV is present on this stud farm and clinical signs of EAV were not observed, but some horses were detected to be shedding EAV. In this situation, a control and vaccination program should produce enough EAV negative pedigree stallions and eradication of the disease in a few years.

References

BRYANS, J. T., E. R. DOLL, M. E. W. CROWE, W. H. McCOLLUM (1957): The blood picture and thermal reaction in experimental viral arteritis of horses. Cornell Vet. 47, 42-52.

CHIRNSIDE, E. D., A. A. F. de VRIES, J. A. MUMFORD, P. J. M. ROTTIER (1995): Equine arteritis virus-neutralizing antibody in the horse is induced by the determinant on the large envelope glycoprotein GL. J. Gen. Virol. 76, 1989-1998.

DUTHIE, S., H. MILLS, P. BURR (2008): The efficacy of a commercial ELISA as an alternative to virus neutralisation test for the detection of antibodies to EAV. Equine Vet. J. 40, 182-183.

FUKUNAGA, Y. R., T. WADA, T. MATSUMURA, H. SUGIURA (1991): Induction of immune response and protection from EAV by formalin inactivated-virus vaccine for EAV in horses. J. Vet. Med. 37, 135-141.

GIBERT, S. A., P. J. TIMONEY, D. DERGET (1997): Detection of equine arteritis virus in the semen of carrier stallions by using a sensitive nested PCR assay. J. Clin. Microbiol. 35, 2181-2183.

GLASER, A. L., A. A. F. de VRIES, E. J. DUBOVI (1995): Comparison of equine arteritis virus isolates using neutralizing monoclonal antibodies and identification of sequence changes in GL associated with neutralization resistence. J. Gen. Virol. 76, 2223-2233.

GLASER, A. L., E. D. CHIRNSIDE, M. C. HORZINEK, A. A. De VRIES (1997): Equine arteritis virus. Theriogenology 47, 1275-1295.

GUTHRIE, A. J., P. G. HOWELL, J. F. HEDGES, A. M. BOSMAN, U. B. BALASURYA, W. H. McCOLLUM, P. J. TIMONEY, N. J. McLACHLAN (2003): Lateral transmission of equine arteritis virus among Lipizzaner stallions in South Africa. Equine Vet. J. 35, 596-600.

HORNER, G. W. (2004): Equine viral arteritis control scheme: a brief review with emphasis on laboratory aspects of the scheme in New Zealand. N. Z. Vet. J. 52, 82-84.

HUNTINGON, P. J., A. J. FORMAN, P. M. ELIS (1990): The occurrence of equine arteritis virus in Australia. Aust. Vet. J. 67, 432-435.

MANKOČ, S., P. HOSTNIK, J. GROM, I. TOPLAK, I. KLOBUČAR, M. KOSEC, D. BARLUČ-MAGANJA (2007): Comparison of different molecular methods for assessment of equine arteritis virus (EAV) infection: A novel one-step MGB real-time RT-PCR assay, PCR-ELISA and classical RT-PCR for detection of highly diverse sequences of Slovenian EAV variants. J. Virol. Methods 146, 341-354.

McCOLLUM, W. H. (1976): Studies of passive immunity in foals to equine viral arteritis. Vet. Microbiol. 1, 45-54.

MITTELHOLZER, C., I. JOHANSSON, A. K. OLSSON, M. RONEUS, M. KLINGEBORN, S. BELAK (2006): Recovery of Swedish Equine arteritis viruses from semen by cell culture isolation and RNA transfection. J. Virol. Methods 133, 48-52.

ST-LAURENT, G., G. MORIN, D. ARCHAMBAULT (1994): Detection of equine arteritis virus following amplification of structural and nonstructural viral genes by reverse transcription-PCR. J. Clin. Microbiol. 32, 658-665.

SZEREDI, L., A. HORYAK, V. PALFI, T. MOLNAR, R. GLAVIS, B. DENES (2005): Study on the epidemiology of equine arteritis virus infection with different diagnostic techniques by investigating 96 cases of equine abortion in Hungary. Vet. Microbiol. 108, 235-242.

TIMONEY, P. J., W. H. McCOLLUM, A. W. ROBERTS, T. W. MURPHY (1986): Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. Res. Vet. Sci. 41, 279-280.

TIMONEY, P. J., W. H. McCOLLUM, T. W. MURPHY, A. W. ROBERTS, J. G. WILARD, G. D. CARSWELL (1987): The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. J. Reprod. Fertil. Suppl. 35, 95-102.

TIMONEY, P. J. W. H. McCOLLUM, T. W. MURPHY (1987): The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the veneral mode of virus transmission. J. Reprod. Fertil. 35, 95-102.

TIMONEY, P. J., W. H. McCOLLUM (1988): Equine viral arteritis - epidemiology and control. J. Equine. Vet. Sci. 8, 54-59.

TIMONEY, P. J., W. H. McCOLLUM (1993): Equine viral arteritis. Vet. Clin. North. Am. Equine Pract. 2, 295-309.

TIMONEY, P. J., W. H. McCOLLUM (2000): Equine viral arteritis: further characterization of the carrier state in stallions. J. Reprod. Fertil. 56, 3-11.

Received: 18 December 2009
Accepted: 9 July 2010

HOSTNIK, P., S. MANKOČ, I. TOPLAK, I. KLOBUČAR, T. MALOVRH, J. GROM: Kontrola arteritisa konja na jednoj ergeli. Vet. arhiv 81, 175-186, 2011.

SAŽETAK

Prikazana je epizootiologija arteritisa konja na jednoj ergeli s 350 konja u razdoblju od 1995. do 2008. godine. Zaraza je bila dokazana na osnovi serološke pretrage virus neutralizacijskim testom (VNT), izdvajanja i identifikacije virusa te RT-PCR-om u sjemenu pastuha. Klinički znakovi bolesti nisu bili primijećeni. Najveća seroprevalencija (gotovo 100%) bila je dokazana u pastuha i starih kobila dok je seroprevalencija u ždrjebica prije pripusta bila manja od 9%. Visoka incidencija serokonverzije bila je dokazana u ždrjebica nakon pripusta. Virus je bio dokazan RT-PCR-om i izdvojen iz sjemena 40,7% od 76 serološki pozitivnih pastuha. Osam pastuha koji su izlučivali virus arteritisa bilo je zaraženo u prvim trima godinama života, a ostalih 12 serološki pozitivnih u kojih virus nije bio izdvojen iz sjemena postali su prvi put serološki pozitivni pet godina nakon ždrijebljenja. Potvrđeno je da se virus u najvećoj mjeri prenosio s pastuha koji su izlučivali virus na ždrjebice za vrijeme pripusta. Za prijenos virusa važan je bio i izravan dodir među različitim skupinama životinja. Pastusi pozitivni na virus bili su kastrirani te je osnovana nova uzgojna jedinica za ždrebad. Ždrebad negativna na virus arteritisa bila je cijepljena, a do treće godine držana izvan ergele.

Ključne riječi: virus, arteritis konja, prijenos, dijagnoza, konj, iskorjenjivanje