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SEROSURVEILLANCE OF EQUINE INFECTIOUS ANAEMIA IN A REGION OF VOJVODINA

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Abstract

Equine infectious anemia is a consequence of a persistent infection of the horses with Lentivirus. Pathogenesis of the disease is very variable, what can be seen through a wide range of clinical forms of the disease – from inapparent infection to death. Diagnostics of EIA is based on clinical symptoms, detection of antibodies and virus. Antibodies can be identified with HI, VN, CFIT, cELISA, SA-ELISA and AGID test. RT-PCR technique enables the detection of and/or quantification of viral RNA level in blood of infected animal. First reliable serological test for EIA was AGID test. Modified AGID test is considered today as acknowledged, international standard for the detection of antibodies against EIA virus and it enables detection of more than 95% of all positive animals. Horses with positive findings with this test are considered infected and should be euthanized or placed in strict isolation. Further measures to control the spread of this disease are insect-vector control and disinfection of surgical and other equipment in use on successive animals. The results of a study during a twenty year period, in the region of AP Vojvodina show that from the total of 11.972 horses blood samples, with the use of AGID test, positive results were found in 21 or 0,17% of horses.

Key words: Equine infectious anemia, horses, seroprevalence, AGID test

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ISPITIVANJE RAŠIRENOSTI INFEKTIVNE ANEMIJE KOPITARA NA PODRUČJU VOJVODINE

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Kratak sadržaj

Infektivna anemija kopitara je posledica perzistentne infekcije konja lentivirusom. Patogeneza IAK je vrlo promenljiva, što se reflektuje kroz širok spektar kliničkih formi bolesti – od inaparentne infekcije do uginuća. Dijagnostikovanje IAK bazira na kliničkim znacima, detekciji antitela i virusa. Antitela se mogu utvrditi pomoću HI, VN, CFIT, CELISA, SA-ELISA i AGID testa. RT-PCR tehnika omogućuju detekciju i/ili kvantifikaciju nivoa virusnih RNK u krvi inficirane životinje. Prvi pouzdani serološki test za IAK bio je AGID test, a modifikovani AGID test se danas smatra priznatim internacionalnim standardom za detekciju antitela za EIAV i moguća je detekcija > 95% svih pozitivnih životinja. Konji sa pozitivnim serološkim testom smatraju se zaraženim, moraju se neškodljivo ukloniti i držati strogo izolovano. Kontrola vektora i dezinfekcija hiruških instrumenata i druge opreme su mere koje se primenjuju u sprečavanju širenja ovog oboljenja. Rezultati ispitivanja tokom dvadesetogodišnjeg perioda na području AP Vojvodine, ukazuju da su od ukupno 11972 uzorka krvnih serum konja, primenom AGID testa, pozitivni nalazi utvrđeni kod 21, ili 0,17% konja.

INTRODUCTION

Equine infectious anaemia (EIA) is a persistent viral infection of equidae. The causative agent, EIA virus (EIAV) belongs to *Lentiviruses* from family *Retroviridae*, subfamily *Orthoretrovirinae*. Equine infectious anaemia occurs in cases of persistent *Lentivirus* infection of horses. The disease is spread out in the whole world and occurs in horses, ponies, mules and donkeys. Clinical cases of the disease was described for the first time in France in the middle of 19th century and viral etiology was identified in 1904 (Timoney et al., 1988). During the following years there was no progress in the introduction of pathogenesis of the disease, only in epidemiology, clinical manifestation of the disease and pathology. All attempts for the virus to be transferred to different experimental

animals, except horses, were unsuccessful (Cook et al. 1996). A reliable diagnostic method was not introduced until 1970 (Coggins and Norcross, 1970). On the contrary to that, during the last few years a breakthrough was done in analysing biological and biochemical characteristics of EIA virus and its pathogenesis.

EPIZOOTIOLOGY

EIA virus can be found in many countries in the world, at all continents. The disease can be found in horses in Europe and the level of incidence depends of the density of the equine population, presence of the vectors and also different programs for control of this disease (Sellon, 1993; Toma, 1980). Equine infectious anaemia is found also in USA, Canada and Latin America, where a high level of incidence was found (Cook et al. 1996; Hall et al., 1988; Timoney et al., 1988). The most important way of infection with EIA in nature is with contaminated blood, mostly transferred by blood sucking arthropods (Kemen and Coggins, 1972). The virus is spread via interrupted feeding of bloodsucking horseflies on a clinically ill horse and then on susceptible horses. Transmission can also occur by the iatrogenic transfer of blood through the use of contaminated needles, because the virus can remain vital up to 4 days (Cook et al. 1996).

Epidemiological proof, with a fact that EIA virus cannot replicate in mammals other than equines, (Kemen and Coggins, 1972) shows that persistently infected horses, ponies, donkeys and mules represent the only reservoir of the virus in the nature. Besides, according to the known data so far, wild type of EIA virus will not replicate in any kind of insect or insect cell lines (Foil and Issel, 1991). That is why it is considered that arthropod transmission of the virus is only mechanical (Kemen and Coggins, 1972). Successful mechanical transmission of the virus depends on several factors such as level of the virus in blood or tissues of the host (titer), characteristics of the vector and its behaviour, behaviour of the host, climate, close presence of the woods, shelters (most of the *Tabanidae* do not enter indoor spaces).

PATHOGENESIS AND CLINICAL SYMPTOMS

Pathogenesis of EIA virus is very variable, caused by the characteristics of the host and the virus, which reflects in a wide range of clinical forms of the disease, from inapparent infection to death. Two important parameters in pathogenesis of EIA are a lifetime persistence of EIA virus in infected host with a spo-

radic occurrence of the disease. Life time persistence of the virus comes from a capability of the virus to intergrete itself into a hromosome DNA of the host. Proviral DNA in integrated and non intergrated state can easily be detected during the period of acute infection. Yet, specific sequences of the virus cannot be detected in asymptomatic horses which indictes that the number of cells which contain inergrated DNA viruses is accually low (Rice et al., 1989). In special conditions such as immunosupression (Dreguss and Lombard, 1954), the reappearance of clinical symptoms is conditioned with the characteristics of EIA virus – it can mutate and make new demands for the immune system of the host.

The disease is characterised by recurrent febrile episodes, thrombocytopenia, anaemia, rapid loss of weight and oedema of the lower parts of the body. If death does not result from one of the acute clinical attacks, a chronic stage develops and the infection tends to become inapparent. The incubation period is normally 1– 3 weeks, but may be as long as 3 months. The severity of the disease varies and it can be in a asymptomatic form or in a form with a high morbidity rate, even with fatal ending. Factors of the host and virus which influence this variability are still not completely clear.

Clinical manifestation of EIA disease can be acute or chronic and there is also the inapparent state of the carrier. Acute form of the disease is often connected with the primary infection and clinical symptoms include pyrexia, anorexia, depression and petechial bleeding of mucosa. Anaemia is not characteristic in acute infections, except in very severe cases when there can also be seen epistaxys and ventral oedema. In chronic infections cycles of healing and return of the disease can be seen with classic symptoms of anemia, edema and weight loss. Death of the animal can occur 4 week later at the earliest after the infection. If the animal lives through the acute phase, the frequency and the severity of the clinical episodes progressively drops (90% can be seen during the first zear after the infection), until the animal becomes an inaparent reservoir of pathogens (Timoney et al., 1988.) Nevertheless, some animals serologically positive to EIA virus, never had any clinical symptoms, or they were in uch a mild form that they could not b noticed by the owner (Issel and Coggins, 1979).

DIAGNOSTICS

Diagnostics of EIA is based on clinical symptoms , analysis of fagocytic blood cells which contain ingested erythrocytes (sideroleucocytes / which are not pathognomonic for EIA, but often are present in acute infection), detecti-

on of antibodies against viral components and detection of the virus. Diagnostics based on clinical symptoms is complex because of the variability of the symptoms and the existence of inapparent carriers. For field strains of EIA virus which replicate only in monocytic/macrophage cells, detection of the virus can be done with a transfusion of 250ml of whole blood (collected in acid-citrate solution of dextrose) from a horse suspected for EIA to a seronegative horse as a receiver. RT-PCR technique enables detection and/or quantification of the viral RNA level in blood of infected animal.

Antibodies can be detected by the following tests: HI, VN, CFIT, cELISA, SA-ELISA and AGID test. The first reliable serological test for EIA was the AGID test (Coggins and Norcross, 1970). However, there are reports that in infected horses can be gained negative findings and what is even more important, some of them can spread the virus (Toma, 1980). Detection in these animals requires more sensitive serological tests. Numerous modifications of ELISA method have been described so far with a p26 as antigen. Competitive ELISA method (cELISA) uses mAb for p26 and ELISA with synthetic antigen (SA-ELISA) can also be used. When using ELISA methods, false positive findings can be expected. Although cELISA and SA-ELISA will detect antibodies somewhat earlier and at lower concentrations than the AGID test, positive ELISAs have to be confirmed using the AGID test (Lew et al., 1993). The AGID test also has the advantage of distinguishing between EIA and non-EIA antigen-antibody reactions by lines of identity. Agar gel immunodiffusion (AGID) tests (Coggins et al., 1972) and enzyme-linked immunosorbent assays (ELISAs) (Suzuki et al., 1982) are accurate, reliable tests for the detection of EIA in horses, except for animals in the early stages of infection and foals of infected dams. In rare circumstances, misleading results may occur when the level of virus circulating in the blood during an acute episode of the disease is sufficient to bind available antibody, and if initial antibody levels never rise high enough to be detectable (Toma, 1980).

More advanced diagnostics is possible with the use of RT-PCR technique, which enables detection and/or quantification of the viral RNA level in blood (Nagarajan and Simard 2001).

OUR INVESTIGATION

During a period of twenty years, blood serum samples of horses were analysed, from two different epizootical regions – from southern Backa and Srem region. Horses originated from two stables, several horse clubs, private owners and one collection point where horses were in quarantine before tran-

sportation for exportation.

Agar-gel immunodiffusion test was used (Coggins test-VMRD Inc.) and in total 11.972 horse serum samples were analysed.

Findings after the analysis of blood serum samples from horses, for equine infectious anemia are shown in Table 1. In the period of study from the total of 11.972 horse serum samples, positive results were found in 21 animal, which is 0,17%.

Table 1. Finding of blood serum samples from horses analysed for equine infectious anemia during the period 1994- 2013

Year	No of horses analysed	No of horses positive for EIA
1994	475	3
1995	312	0
1996	508	1
1997	421	1
1998	214	0
1999	184	2
2000.	593	0
2001.	1359	0
2002.	446	0
2003.	593	1
2004.	381	2
2005.	1065	1
2006.	417	0
2007.	874	0
2008.	425	0
2009.	522	1
2010.	611	0
2011.	826	0
2012.	957	6
2013.	789	3
TOTAL	11.972	21 (0,17%)

DISCUSSION

Equine infectious anaemia can cause damage and it can be a deadly disease. Until today, treatment for horses that have EIA is not known. Also, there is no vaccine on the market against EIA virus for the protection of horses. But with control measures and management techniques that are strict and appropriate for implementation in horse breeding, the chances of infection with EIA virus can be pretty much reduced.

Horses infected with EIA virus represent a danger and threat to the community. The consequences can be of different levels and risk from EIA is still to be estimated. In order to prevent the spreading of EIA virus, strict control measures should be applied (Cook et al. 1996). In some rural areas horses still participate in everyday life, taking part in agriculture and transportation. A decrease of the potential risk of great economical losses resulting from infectious diseases occurring in horses is very important.

A significantly higher number of infected animals has been found in Greece. During the period 2001-2008, a total of 7.872 horse serum samples were tested at the Centre of Veterinary Institutes of Athens. Antibodies against equine infectious anaemia (EIA), were found in 4.5% of the samples and seropositivity for EIA was determined (Mangana-Vougiouka et.al. 2013).

To the contrary of this, there are data from several regions in Turkey. A study was done where the material consisted of 8.947 horse serum samples, including 8.769 horses and 178 donkeys, from Ardahan, province in north east part of Turkey (Albayrak and Ozan, 2010). Blood was collected from all horses and donkeys and the sera were analysed for the presence of antibodies for equine infectious anaemia virus (EIAV) using an enzyme-linked immunosorbent assay. All animals were negative for antibodies against EIA virus. EIA infections are also reported in different countries (Pearson and Knowles 1984; Lew et al. 1993; Nagarajan and Simard 2001). In Armenia and Georgia EIA has not been reported so far, but the presence of haematophagous vectors as important risk factors of EIA for equines has been found (Erdem, 2007). Since 1981, in Serbia there is a program for prevention and, eradication of EIA in horses (Vidić et. al. 1998.). EIA is a disease that is mandatory for reporting to the OIE and disease which is under annual program of monitoring, administered by the Ministry of Agriculture and environment protection of Serbia.

Gained results show that EIA is present in horses from the region included in the study and there is a very low prevalence. There is no tendency of the virus to spread and we can assume that the gained results are a consequence of a horse trade market, because EIA was found in horses from other regions of Serbia, intended for slaughter.

PROPHYLAXIS AND CONTROL

An attenuated live vaccine, developed in the early 1970s, was extensively used in China (OIE Terr. Manuel, 2008) between 1975 and 1990. Until today, there is no available vaccine against EIA virus, except in China, because of the complex development of the vaccine which is conditioned by the life cycle and

antigen performances of *Lentivirus*. Control measures for EIA have the aim to reduce the probability of the appearance of infection. This is achieved by the good management on the farm, strict laws and regulations and the capability of detecting and separation of infected animals. Good practice on farms involves separation of infected animals, grazing animals far from the edge of the woods, shelter from the tabanides attack, usage of spray and repellents for reduction of the vectors and also the usage of the proper procedures of vaccination or blood sampling can help in the prevention of the spreading of EIA. Besides all this, most of the countries have their own regulations for EIA with the measures “test and remove”, which limit the breeding and trade of the infected animals. All of the horses with positive finding are considered infected and should be euthanized or placed in strict isolation. Further measures to control spread of this disease are insect-vector control and disinfection of surgical and other equipment between use on successive animals. These regulations include the obligation of permanent identifikation of infected animals (lip tatoo, stamps, electronic implants).

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