

Equine Viral Arteritis Prevalence in Eastern Part of Romania, Impact Study Imposed by Uncontrolled Breeding Process

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Abstract: The research was realized on a number of 406 samples collected from equine population located in Eastern part of Romania. Samples were tested using enzyme linked immunoassay in order to evidence antibodies against equine arteritis virus. Seroprevalence varied depending on region.

Key words: Equine arteritis virus, ELISA, seroprevalance, antibodies, Romania

INTRODUCTION

Equine viral arteritis called also typhoid fever is an infectious, contagious disease specific to soil hoofed family, characterized by fever, deep depression, nasal and ocular catarrh edemas in subcutaneously tissue, jaundice and abortion.

Up to 1964 the disease was limited to USA territory later being signaled in European states, too (Switzerland and France). Due to the fact that lack of diagnostic capabilities this disease was not identified with certitude, on the American continent; the predominant form of equine viral arteritis was the abortive one whereas in Europe the dominant form was the typhoid one (Bryans *et al.*, 1957).

In Romania, the disease was signaled for the first time by Aurelia Ionescu in 1993 later on were reported viral arteritis episodes in almost all studs with a varied clinical expression (Ionescu, 2006).

In economic terms of value the losses are important, they are caused mainly by abortion, reproduction disorders, convalescence costs, surveillance and disease control.

Equine arteritis is caused by a virus fitted taxonomically in *Arterivirus* genus, Arteriviridae family. Arteriviruses are small, enveloped with an icosahedral core containing a positive sense RNA genome. The virus has a unique antigenicity and virulence which differs depending on isolated strain.

Using sequential analysis of the genes that code the virus envelope and capsid, the strains were divided in two phylogenetic groups (Balasuriya *et al.*, 1998). The relations between the viral strains are useful in molecular epidemiology in order to establish focal traceability.

To the natural infections are susceptible only the equine populations of any age, gender and or physiologic status (Holyoak *et al.*, 2008).

The transmission is realized by airway, sexual and transplacental contamination (McCollum and Timoney, 1999). The most important contamination sources are the abortive tissues, fetal liquids, genital secretions of the infected mare and the semen. The stallions remain carriers for this virus up to several months, sometimes for the rest of their life. Semen conveys infection abroad by natural mating or artificial insemination, the virus is not inactivated by low temperatures used for semen preservation. The virus is located in stallion accessory glands being eliminated during ejaculation.

Despite the fact is seropositive, a stallion does not always eliminates the virus but vice versa is always true, all stallions that eliminates the virus through semen being diagnosed seropositive.

If passage is airborne the virus multiplies primarily in alveolar macrophages from where passes immediately to bronchial lymph nodes. From this level enters into blood stream, being conveyed to all systems (Timoney and McCollum, 2000). Into the next phase the virus accumulates in accessory glands, testes, thyroid, kidney and liver. It is considered that virus multiplication is stimulated by steroid hormones, fact proven by the fact that the virus was not evidenced in stallions infected then castrated.

Epidemiological data, clinical evolution and pathological exam allow only a presumptive diagnostic, confirmed by laboratory findings (virological, serologic and histological exam) (Del Piero *et al.*, 1997; Ruiz-Saenz, 2010).

The virus is isolated on cellular cultures and identified using sero-neutralisation or direct immunofluorescence. The second test can be used directly on samples collected for diagnostic from suspect horses.

Samples collected in/for diagnostic purpose are: scrapings from nasal and pharyngeal mucosa, blood collected on citrate, semen, aborted fetuses (lymph nodes and lungs), placenta and fetal liquids (Ruiz *et al.*, 2010). In persistent infections only semen is used for virus isolation because other samples contain the virus in early weeks of disease. Cellular cultures used for virus isolation are primary equine or rabbit cellular lines as RK 13 VERO or LLC-MK2 (Nugent *et al.*, 2000).

The diagnostic can be confirmed retrospectively, identifying specific antibodies presence and rise, (Cho *et al.*, 2000). Serums are checked using ELISA assay or indirect immunofluorescence (Bulut *et al.*, 2012).

Histological exam provides valuable information in diagnostic confirmation. Vasculitis has a high diagnostic value even pathognomonic, present in small arteries from spleen, colon, cecum, lung, kidney, lymph nodes. Hyalinosis of the myocytes from the media of these vessels is present.

MATERIALS AND METHODS

The researches were realized on samples collected from 406 equines selected from nonprofessional exploitation, situated in six counties from eastern part of Romania. The selection of these effectives is based on the fact that during past years, these populations have confronted with an abortion increase.

Samples distribution on administrative units is: Botosani county 15 samples, Vaslui county 114 blood samples, Galati county 57 samples, Neamt county 84 samples and Bacau county 72 samples. The individuals constituting this workgroup are between 46 and 138 months of age, females and males as well.

Researchers used indirect ELISA test for titrating specific antibodies against viral equine arteritis. This test is a confirmation test produced by ID-VET and the work procedure followed the recommendations of the producer.

RESULTS AND DISCUSSION

From 406 serums analyzed, samples harvested from six counties, only 129 have reacted positive fact representing a prevalence of 31.77% (Table 1).

Subsequently, ELISA testing it can be observed the fact that in Iasi county were 17 positive reactions representing prevalence of 26.56% and in Botosani county reacted positive only 2 samples of 15 analyzed,

Table 1: Results on administrative regions

Administrative region	Results				Total samples analysed
	Positive samples		Negative samples		
	No.	%	No.	%	
Iasi	17	26.56	47	73.44	64
Botosani	2	13.33	13	86.67	15
Vaslui	44	38.59	70	61.41	114
Galati	16	28.07	41	71.93	57
Neamt	29	34.52	55	65.48	84
Bacau	21	29.16	51	70.84	72
Total	129	31.77	277	68.23	406

representing a prevalence of 13.33%. In the other 4 administrative regions prevalence was higher in Vaslui county seroprevalence was 38.59% representing 44 positive samples of 114 analyzed in Galati the prevalence was 28.07% (16 positive samples of 57 samples analyzed) in Neamt the prevalence was 34.52% (29 positive samples from a total of 84 samples analyzed) finally in Bacau county there was a positivity of 29.16% (21 positive samples from 72 analyzed).

Considering seroprevalence on regions the lowest one was encountered in Botosani county (13.33%) and the highest one in Vaslui county (38.59%).

All six administrative regions are situated in the eastern part of Romania and all horses belong to private exploitations being held on pasture during heat season and the reproduction process is not supervised (without origin book, the animals originates from local breeds and lines).

From specialty literature is known the fact that except aerial infection the way venereal one represents the main infecting route, stallions excrete the virus by semen, infecting the mare during sexual intercourse (Ionescu, 2006).

This is why there is recorded a rise in abortion rate during past years. In stallions virus distribution at different levels of the reproductive tract depends by physiological status or age.

Research team represented by Little *et al.* (1992) from Gluck Equine Research Center, Kentucky University), proven the fact that the virus persistence in stallion is dependent on testes presence and mediated by testosterone. Removal of the testes leads to loss of seropositive status in one up to three months. The virus is located at the level of seminal ampullas, deferent ducts and in accessory glands usually the spermatic fraction of the ejaculate contains high amounts of virus. The virus does not have been identified in seminal fluids.

In the animals kept on pasture the most incriminated way of transmission is the airborne and the oral one. In Romania the serological surveillance by ELISA assay and seroneutralization is done in stallion studs and in mares

(20% of them) once a year. In case of suspicion serological exams are carried out on twin samples at 21 day interval, virological exams are performed to.

It can be observed the fact that serological surveillance is applied only in horse studs and in animals important for breeding process.

The higher prevalence registered in the past years can be explained by the better diagnostic capabilities and breeders uncontrolled circulation. There is obvious that is necessary to implement programs in order to limit contact between individuals, the untested horses with an uncertain status.

CONCLUSION

The most important factor in disease dissemination is represented by stallions uncontrolled serologically. Because this virus is widely spread, serological surveillance is a demanding priority for animals that are subject to sale, circulation in public areas, reproduction and sportive events. Considering the fact that in Romania there are no vaccination programs all immune responses are consecutive to natural infection. Resistance to this virus is dependent on housing and forage quality, the breed has a reduced role.

ACKNOWLEDGEMENT

The research was done thanks to research project PD-375/2010 obtained by Asistant Dr. Tanase Oana Irina from Romania CNCISIS.

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