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## ■ Serological investigation of Equine Viral Arteritis (EVA) in Bulgaria

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## ■ Ορολογική διερεύνηση της ιογενούς αρτηρίτιδας (EVA) των ιπποειδών στη Βουλγαρία

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**ABSTRACT.** The aim of the present study was to estimate the existence of EVA infection in equine's population in Bulgaria. For this purpose the clinical signs of the disease, as well as the antibodies titre against the virus in unvaccinated equines population, were estimated. Out of 1.743 tested non vaccinated animals, 1.425 blood sera were positive for antibodies to EVA, 81.76% positivity, during 2003 - 2007. The positive samples were coming from 14 stables, riding clubs and breeding farms of the country. EVA is considered as a major problem of veterinary importance in Bulgaria during the current and next years.

**Keywords:** Equine viral arteritis, epizootiology, clinical signs, ELISA

**ΠΕΡΙΛΗΨΗ.** Σκοπός της παρούσας έρευνας ήταν να εκτιμηθεί η παρουσία της EVA λοίμωξης στα άλογα της Βουλγαρίας. Για το λόγο αυτό μελετήθηκαν και αξιολογήθηκαν τα κλινικά συμπτώματα της νόσου και το επίπεδο των αντισωμάτων κατά του ιού σε μη εμβολιασμένα άλογα. Από τα 1.743 μη εμβολιασμένα άλογα που εξετάστηκαν, 1.425 δείγματα ορού αίματος ήταν θετικά για τα αντισώματα EVA, δηλαδή ποσοστό 81.76% θετικότητα, κατά τη διάρκεια 2003-2007. Τα θετικά δείγματα προέρχονταν από 14 στάβλους, λέσχες ιππασίας και εκτροφές αναπαραγωγής από όλη τη χώρα. Η Ιογενής αρτηρίτιδα των ιπποειδών (EVA) θεωρείται ως ένα μέγιστο πρόβλημα κτηνιατρικού ενδιαφέροντος στη Βουλγαρία κατά τη διάρκεια αυτών και των επόμενων χρόνων.

**Λέξεις ευρετηγίας:** Ιογενής αρτηρίτιδα ιπποειδών, επιζωοτιολογία, κλινικά συμπτώματα, ELISA

## INTRODUCTION

Equine viral arteritis (EVA) has considerable global significance, having been identified in many countries of the EU and in America. Serological investigation of the disease indicates that the infection occurs in other areas of the world, as well. The major

consequences of EVA are that the infection may be abortigenic and that a significant percentage of infected stallions recover clinically, but became long-term venereal "carriers" of the virus (Clayton, 1986; Glaser et al., 1997; Mumford, 1985; Timoney et al., 1993; Timoney, 1999, 2000).

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**Table 1.** Samples tested positive for EVA sera in the fourteen regions in Bulgaria using ELISA.

Stable number	Horses up to 2 years old		Horses from 2 to 5 years old		Adult stallions and mares		All equines	
	All	+	All	+	All	+	All	+
1	16	12	6	6	24	24	46	42
2	6	6	7	7	12	11	25	24
3	40	30	90	50	110	100	240	180
4	15	4	15	11	80	60	110	75
5	14	-	24	2	28	15	66	17
6	50	50	60	56	58	42	168	148
7	25	24	50	40	41	30	116	94
8	30	28	90	82	80	76	200	186
9	40	40	100	90	100	96	240	226
10	29	26	100	92	57	57	186	175
11	20	20	40	36	40	32	100	88
12	10	8	50	40	51	30	101	78
13	10	8	24	20	22	16	56	44
14	12	8	20	18	18	16	50	44

Direct economic consequences of equine arteritis virus infection are due to: 1) incidence of abortion or death of very young foals; 2) prohibited export markets for carrier stallions, virus infected semen and other categories of naturally seropositive horses; and 3) reduced commercial value of carrier stallions and reduced demand to breed these animals. Up to 2001 the disease was not referred in Bulgaria (Chenchev et al., 2002).

Equine arteritis virus belongs to the *Arterivirus* genus of the family *Arteriviridae* (Doll et al., 1957; Cavanagh et al., 1997; Glaser et al., 1997). The viral particles are 60 nm in diameter and consist of an isometric nucleocapsid surrounded by a lipid envelope. The genome of the virus consists of a single molecule of linear, positive-stranded RNA about 12.7 Kb in size and comprising multiple open reading frames. The ORFs encode seven polyadenylated RNAs, which are translated into proteins. Virions are composed of four major structural proteins: a nucleocapsid protein, surrounding the genomic RNA, an integral membrane protein and two N-glycosylated surface glycoproteins. In addition, the genome codes for a viral polymerase and two non-structural proteins of unknown function.

The aim of the present study was to estimate the existence of EVA antibodies in Horse population in Bulgaria as no vaccination policy was applied. Prophylactic measurements against the disease should be developed.

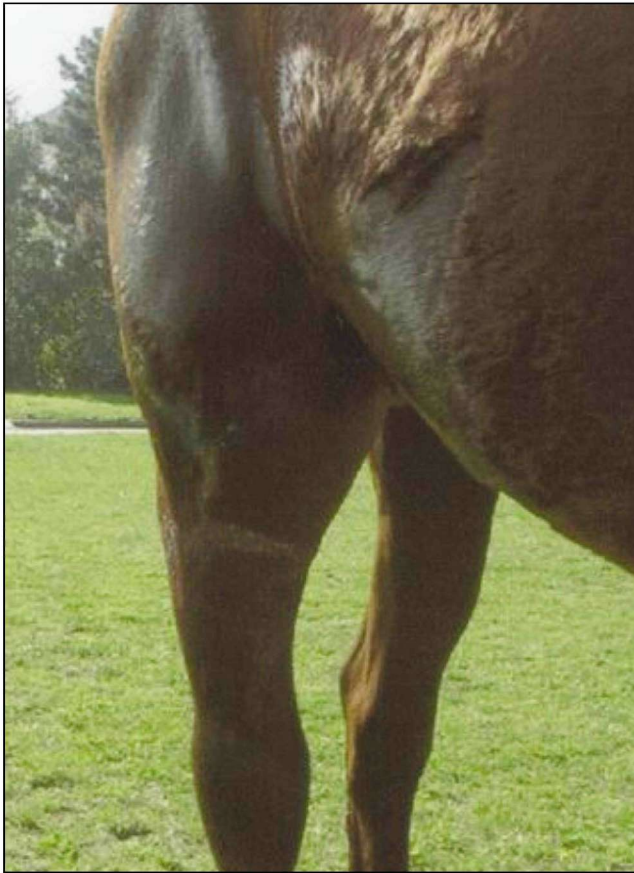
## MATERIALS AND METHODS

The first cases of clinical disease with reproductive failure and heavy respiratory syndrome was observed during February 2003 in three horse-riding clubs with 128 animals. The same clinical picture was noticed during spring - summer of the same year in other horse units.

During that period the study was carried out in 14 stables. Eight of them were belonging to horse riding private clubs with 5-25 animals; two were breeding farms with 80-120 and four were private big stable with 180-350 animals. The farms were situated in fourteen regions of the country.

The sampling period was from February 2003 to December 2007. The blood samples were taken from different categories of horses after 14th day from the first clinical signs. Blood samples were collected and sera were kept at -20°C, until tested using ELISA test (/Ingenasa-Madrid).

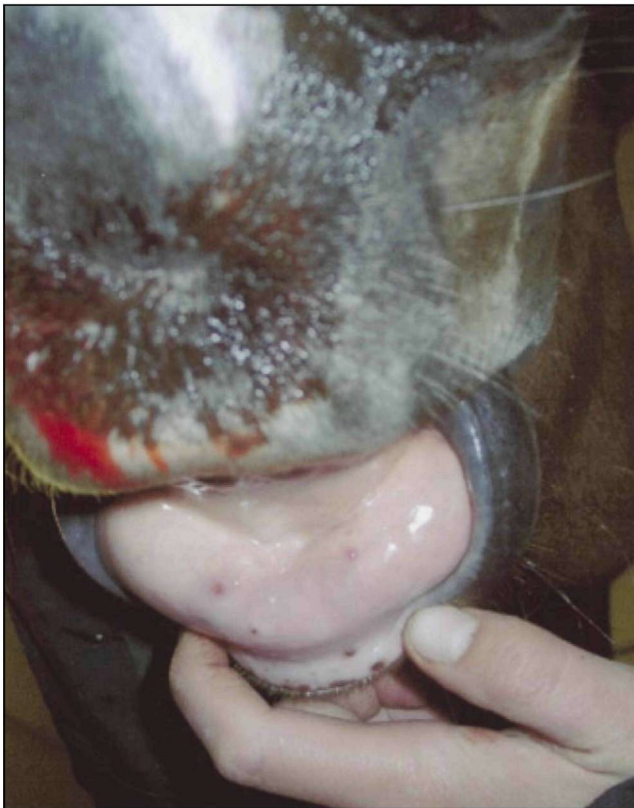
Difference in serological response were analysed by mixed model (VENABLE and RIPLEY 2002) on a logarithmic transformation of titres, according to formula  $\log(\text{titre} + 1)$ , in order to take account of zero value of negative results. In the model the "Day of First examination"/DF/ and the "Day 14th after clinical signs"/D14CS/. / The dates of EAV ELISA 3.78(0.71)/. All statistical analysis and plot were made with statistical software R (R Development Core Team 2003).



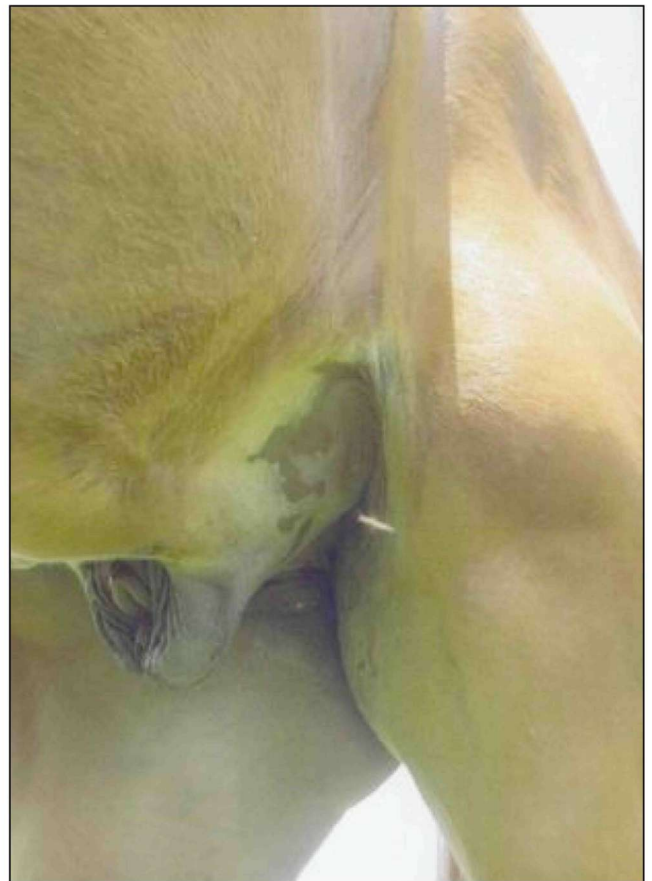
**Figure 1.**  
Edema of the area  
of buttock.



**Figure 2.**  
Edema in knee.



**Figure 3.** Haemorrhages in the lips and nostril.



**Figure 4.** Edema of preputium.

## RESULTS

The results of ELISA for antibody detection are showed in Table 1. Out of 1.425 tested blood horse sera, 1.425 samples were positive including those from all ages groups of animals, positivity 81.76%.

The linear mixed model for EAV antibodies in

different categories of equines shows a significant interaction between the "Day of First examination" and the "Day 14th after clinical signs" (Figure 1, 2, 3 and 4).

In fact, the comparison between different investigated equine's category shows a reliable mean of log titres, at DF ( $p < 0.05$ ) and at D14CS ( $p < 0.0001$ ).

Indeed, a discrepancy was observed between the two replicates of the same trial /in stable numbers 2 and 5/. In addition, in animals of the second and fifth stable we detected at D14CS a sharp increase of the level of specific antibodies. This situation could be due to a secondary wild infection of the animals with EAV before our testing.

## DISCUSSION

The clinical signs and lesions in different horse categories, that were estimated in total of 14 stables and compared with those described by other researchers, were characteristic for EVA infection (Glaser et al., 1997; Mumford, 1985; Paweska et al., 1993; Timoney 1999, 2000; Holyoak et al., 2008) Stallions may become persistent carriers of the virus and transmit the infection during mating. The duration of

the carrier state can be several years, even for the entire life of an individual stallion.

The detection of antibodies to EVA in unvaccinated animals using ELISA and the negative results for other viral or bacterial infections indicate the circulation of the virus of Equine Viral Arteritis in the Bulgarian horse farms (Clayton et al., 1986; Chenchev et al., 2002; Cho et al., 2000; Glaser et al., 1997; Timoney et al., 1999, Timoney 2000; Cullinane et al., 2008; Westcott et al., 2008).

In conclusion, unvaccinated horses positive in EVA antibodies were detected for the first time in Bulgaria. Laboratory tests are required to diagnose outbreaks of clinical disease, to screen for subclinical infection and to identify carrier stallions, by viral isolation from semen. The disease has to be considered as a major problem of veterinary importance with high economic impact.

In conclusion, a national strategy and the prophylactic scheme have to be developed to control the disease. ■

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