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DETECTION OF ANTIBODIES AGAINST EQUINE VIRAL ARTERITIS VIRUS (EVAV) AND EQUINE HERPESVIRUS TYPE 1 (EHV-1) IN CART HORSES FROM CURITIBA AND SURROUNDINGS, SOUTHERN BRAZIL

(Detecção de anticorpos contra o vírus da arterite eqüina (EVAV) e herpes vírus eqüino tipo 1 (EHV-1) em cavalos de carroceiros de Curitiba e Região Metropolitana, Paraná, Brazil)

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RESUMO – Aproximadamente 1.500 cavalos de carroceiros com baixo acesso à assistência veterinária e seus proprietários trafegam diariamente pela Região Metropolitana de Curitiba para coleta de material reciclável, incluindo papel, metal, plástico e vidro. Doenças virais transmitidas por aerossol são de importância na medicina eqüina de populações expostas que compartilham o mesmo ambiente. O propósito deste estudo foi de avaliar a ocorrência de anticorpos contra o vírus da arterite eqüina (EVAV) e o herpes vírus eqüino tipo 1 (EHV-1) em cavalos de carroceiros de Curitiba e Região Metropolitana. Um total de 97 amostras de sangue foram coletadas de cavalos de carroceiros sem raça definida sendo 51 machos e 46 fêmeas, média de 15,3 anos de idade de abril de 2005 a junho de 2006 em Curitiba e São José dos Pinhais. Todas as 97 amostras testadas foram negativas para EVAV. Entretanto, um cavalo de Curitiba (1/25) e quatro de São José dos Pinhais (4/72) foram positivos para EHV-1, com total de cinco cavalos (4.1%). Os resultados demonstraram taxas mais baixas de EVAV e EHV-1 quando comparados com outros estudos em cavalos puros ou para outros propósitos. Em conclusão, apesar dos cavalos de carroceiros realizarem tráfego diário em centros urbanos e terem pouco acesso à assistência veterinária, estes parecem estar menos expostos ao risco de infecção por EVAV e EHV-1 quando comparados com cavalos utilizados para esportes, exposições e reprodução.

Palavras-chave: Cavalos de carroceiros; EVAV; EHV-1.

ABSTRACT – Approximately 1,500 cart horses with low access to veterinary assistance and their owners traffic daily through Curitiba and surroundings to collect recycling material, including paper, metal, plastic and glass. Viral diseases transmitted by aerosol are of importance in equine medicine of exposed populations which share the common airspace. Accordingly, the purpose of the present study was to evaluate the occurrence of antibodies against equine viral arteritis virus (EVAV) and equine herpesvirus type 1 (EHV-1) in cart horses of Curitiba and surroundings. A total of 97 samples were collected from crossbred cart horses, 51 males and 46 females, with average age of 15.3 years (from 4 months to 22 years) from April 2005 to June 2006, from Curitiba and São José dos Pinhais, Southern Brazil. All 97 samples tested were negative for EVAV. However, one horse from Curitiba (1/25) and four from São José dos Pinhais (4/72) were found positive for EHV-1, performing a total of five horses (4.1%). These results demonstrated lower rates of EVAV and EHV-1 when compared to other studies in purebred horse populations or horses used for other purposes. In conclusion, despite cart horses undergo daily traffic in urban areas with low access to veterinary assistance, they seem less exposed to the infection risk of equine viral arteritis virus and equine herpesvirus type 1 when compared to horses utilized for sports, exposition and reproduction.

Key-words: Cart Horse; EVAV; EHV.

Introduction

Equine viral arteritis virus (EVAV) is caused by a RNA virus from the family *Arteriviridae* and genus *Arterivirus* (CAVANAGH *et al*., 1994). EVAV is an acute viral disease of horses that is characterized by respiratory disease, subcutaneous edema and abortion. Subclinical infections are common and stallions may persistently shed the virus in their semen. With possible exceptions of the British Isles and Japan, the virus has a worldwide distribution (MUMFORD, 1994a). Some presenting clinical signs include fever up to 41°C, apathy, depression, anorexia, edema of pelvic limb extremities, scrotum and mammary gland edema, nasal and ocular exsudative secretion, conjunctivitis and rhinitis (TIMONEY and McCOLLUM, 1988; TIMONEY and McCOLLUM, 1993). Virus transmission from an animal to susceptible horses during acute phase of the disease may occur by respiratory tract, and it appears to be the most frequent transmission

during the disease outbreak (McCOLLUM *et al*. 1971). Stallions may be asymptomatic and infect mares during service (TIMONEY *et al*.,1987).

Sorological studies have been conducted in several Brazilian regions and include Paraná (LARA *et al*. 2003), São Paulo (SOUZA, 1996; SOUZA *et al*., 1999; LARA *et al*., 2002), Pará (HEINEMANN *et al*., 2002) and Rio Grande do Sul (DIEL *et al*., 2006).

Equine herpesvirus type 1 (EHV-1) is a directly transmitted DNA virus, which causes four distinct syndromes in horses: respiratory disease (rhinopneumonitis), abortion, neonatal foal and, more rarely, paresis or paralysis (MUMFORD, 1994b). EHV-1 belong to the family *Herpesviridae* and genus *Alphavirus* (MURPHY *et al*., 1999), are widespread throughout the World (MUMFORD, 1994b) and is one of the most important contagious viral diseases in equine medicine (OSTLUND, 1993). Transmission may occur by aerosol, ingestion or fomites contaminated with viral particules from a sick or asymptomatic horse. In order to be effective, infection requires close contact betwe are contaminated and susceptible horses (KEMEN, 1975). Clinical signs of this viral disease in foals are typically of respiratory form, and include hyperthermia, reaching 41°C, anorexia, enlargement of submandibular lymph nodes, bilateral serous nasal discharge, nasal and ocular mucosa congestion. Clinical signs of respiratory form is rarely observed in adult horses (KEMEN, 1975). The abortive form may occur in mares, with foals born weak and sick and with a highly death rate at the first week of life (MUMFORD 1994b). Adult horses may present also a neurological syndrome, with signs of paresis and paralysis of pelvic limbs and urine / fecal retention (RIET-CORREA *et al*., 2002).

EHV-1 has been isolated in Brazil (NILSSON and CORRÊA, 1966), with serological surveys conducted in some Brazilian States such as Paraná (MOREIRA, *et al*., 2000; LARA *et al*., 2003), Rio Grande do Sul (VARGAS and WEIBLEN, 1991; DIEL *et al*., 2006), São Paulo (FERNANDES, 1988; MODOLO *et al*. 1989; CUNHA *et al*. 2002) and Pará (HEINEMANN *et al*., 2002).

Viral diseases which may be transmitted by aerosol are of particular importance in equine medicine due to the potential risk of outbreaks in horse populations which share the common airspace. Besides, groups of animals with low access to veterinary assistance and routinely traffic in highly horse populated areas may also increase the exposure risk to the agent (MUMFORD 1994b).

Curitiba is the fifth most populated State capital of Brazil. According to the local Center of Disease Control, approximately 1,500 cart horses and their owners, traffic daily through Curitiba and surroundings to collect recycling material, including paper, metal, plastic and glass. Reports of other big cities in Brazil describe similar increase in cart horse recycling activity, and the vast majority of cart horses belong to poor working people who can not afford veterinary assistance. Accordingly, the purpose of the present study was to evaluate the occurrence of antibodies against equine viral arteritis virus (EVAV) and Equine herpesvirus type 1 (EHV-1) in cart horses of Curitiba and surroundings, as well as its potential impact in animal health.

Materials and Methods

Samples were collected from cart horses of urban areas of Curitiba and São José dos Pinhais, Southern Brazil from April 2005 to June 2006. All animals were spontaneously taken to the Center of Disease Control by their owners. A total of 97 crossbreed horses, 51 males and 46 females, with average age of 15.3 ± 4.2 years (from 4 months to 22 years) were included in this study. According to the owners, horses were routinely used for carrying carts of recycling material in Curitiba and surroundings area, and presented no vaccination record.

Blood samples were collected by veinpunction with a vacuum tube system. Sera were separated and stored at -20º C until processing at the Instituto Biológico, São Paulo, Brazil and screened for equine viral arteritis virus (EVAV) and equine herpesvirus type 1 (EHV-1). Standard microplate serum neutralization test was used for both tests, based on the protocol of SENNE *et al*. (1985) for EVAV and of KOTAIT *et al*. (1989) for EHV-1. Standard samples of EVAV, as well as negative and positive controls, were obtained from Dr. Peter J. Timoney, of University of Kentucky, USA. Standard samples of A4/72 virus isolated at Instituto Biológico were used for EHV-1 (REINER, *et al*. 1972). RK-13 cell lineage was used for EVAV and VERO cell lineage for EHV-1, both cultivated in Minimum Essential Medium Eagle (MEM), added with 10% of Bovine Fetal Serum (FBS).

Results

Out of 97 samples tested, no seropositive animal was found for equine viral arteritis virus (EVAV). However, one horse from Curitiba (1/25) and four from São José dos Pinhais (4/72) were found positive for equine herpesvirus type 1 (EHV-1), performing a total of five positive horses (4.1%), as shown on TABLE 1.

TABLE 1 – SURVEY OF SERUM ANTIBODIES TO EQUINE HERPESVIRUS TYPE 1 (EHV-1) IN 97 CART HORSES FROM URBAN AREAS OF CURITIBA AND SÃO JOSÉ DOS PINHAIS, SOUTHERN BRAZIL, ACCORDING TO GENDER AND CITY.

		Positive samples for EHV-1		
Local	Animals	males	females	Total
Curitiba	25	0/13 (0%)	1/12 (8.3%)	1/25 (4.0%)
São José dos Pinhais	72	0/38 (0%)	4/34 (11.7%)	4/72 (5.6%)
Total	97	0/51 (0%)	5/46 (10.9%)	5/97 (5.2%)

Discussions and conclusion

The occurrence rate of antibodies against equine viral arteritis virus (EVAV) in the present study was negative. Similar results were found in Pará, Northern Brazil, were no positive animal was found out of 96 horses from 32 farms (HEINEMANN *et al*., 2002).

However, relative higher occurrence rate of antibodies against EVAV was found: 2.9% in Thoroughbred horses from Curitiba and surroundings (LARA *et al*., 2003), and 1.04% in horses from São Paulo State, Southeastern Brazil (SOUZA *et al*., 1999). Also higher results were found in another survey of antibodies against EVAV in horses used for sports, exposition and reproduction, with prevalence rates of 2.0%, 2.1% and 3.8%, respectively; in the same study positive horses were found in 23% of the 65 cities searched (DIEL *et al*., 2006).

A possible explanation for these contrasting results is that horses utilized for sports, exposition and reproduction may be at higher contact with each other, therefore sharing airspace and facilitating the spread of disease when compared to cart horses and extensive raised horses, as observed in Northern Brazil (HEINEMANN *et al*., 2002).

The occurrence rate of antibodies against equine herpesvirus type 1 (EHV-1) in the present study was 4.1%. Relative higher occurrence rate of EHV-1 was found in two other studies in the same area of Curitiba and surroundings: 14.3% in thoroughbred horses (SOUZA *et al*., 1999), and 17.7% in Thoroughbred, Brasileiro de Hipismo, Hanoverian, Westphalen, Reitpony and Arabian horses with no significant differences found among breeds (MOREIRA *et al*., 2000).

The higher results of other studies when compared with the present study suggest that the raising conditions of purebred horses may be important for the disease mechanism and transmission. Since purebred horses are intensively raised, close to each other in transportation, expositions, auctions and tournaments, it may increase the possibility of contamination through aerosol spreading (KEMEN, 1975; DIEL *et al*. 2006). On the other hand, low income owners who work with recycle materials commonly possess one to few crossbred cart horses, probably reducing the contact risk. Therefore, although these horses transit daily through urban areas, they may have a lower contact with other horses when compared to purebred ones. New techniques such as polymerase chain reaction (PCR) have been applied for survey EHV-1 infection, and may increase the detection sensitivity of this disease (CARVALHO *et al*., 2000).

Studies performed in several areas of Brazil showed different results. In Rio Grande do Sul, Southern Brazil, a prevalence of 84.7% and 4.7% of seropositive animals for EHV-1 was found in two different studies (VARGAS and WEIBLEN., 1991; DIEL *et al*., 2006). Lower rates of EHV-1 prevalence may be due to semi-extensive raising conditions of horses, preventing the spreading and contamination (DIEL *et al*., 2006). Studies in São Paulo, Southeastern Brazil, showed different results: 67.2% (FERNANDES 1988), 17.6% (MODOLO *et al*., 1989), 13.5% (KOTAIT *et al*., 1989) and 27.2% (CUNHA *et al*., 2002).

A study conducted in Pará State, Northern Brazil, demonstrated a 17.7% prevalence of seropositive horses and 40.62% positive farms for EHV-1 (HEINEMANN *et al*., 2002). Interestingly, the same study found no positive animal for EVAV, what shows that the infection may be multifactorial and therefore not restricted to a single risk factor.

Finally, none of these studies were found from horses raised in urban areas and therefore they may not be compared to the cart horses, which are raised and daily transit through urban areas for collecting recycle material. In conclusion, horses utilized for sports, exposition and reproduction may be at higher risk of Equine Viral Arteritis and equine herpesvirus type 1 when compared to cart horses and extensive raised horses.

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