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PRESENCE OF RESPIRATORY VIRUSES IN EQUINES IN BRAZIL

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SUMMARY

Equines are susceptible to respiratory viruses such as influenza and parainfluenza. Respiratory diseases have adversely impacted economies all over the world. This study was intended to determine the presence of influenza and parainfluenza viruses in unvaccinated horses from some regions of the state of São Paulo, Brazil. Blood serum collected from 72 equines of different towns in this state was tested by hemagglutination inhibition test to detect antibodies for both viruses using the corresponding antigens. About 98.6% (71) and 97.2% (70) of the equines responded with antibody protective titers (≥ 80 HIU/25 μ L) H₇N₇ and H₃N₈ subtypes of influenza A viruses, respectively. All horses (72) also responded with protective titers (≥ 80) HIU/25 μ L against the parainfluenza virus. The difference between mean antibody titers to H₇N₇ and H₃N₈ subtypes of influenza A viruses was not statistically significant ($p > 0.05$). The mean titers for influenza and parainfluenza viruses, on the other hand, showed a statistically significant difference ($p < 0.001$). These results indicate a better antibody response from equines to parainfluenza 3 virus than to the equine influenza viruses. No statistically significant differences in the responses against H₇N₇ and H₃N₈ subtypes of influenza A and parainfluenza 3 viruses were observed according to the gender (female, male) or the age (≤ 2 to 20 years-old) groups. This study provides evidence of the concomitant presence of two subtypes of the equine influenza A (H₇N₇ and H₃N₈) viruses and the parainfluenza 3 virus in equines in Brazil. Thus, it is advisable to vaccinate equines against these respiratory viruses.

KEYWORDS: Parainfluenza virus; Influenza virus; Serology; Hemagglutination inhibition test; Equines.

INTRODUCTION

Influenza is a contagious disease caused by negative eight-stranded RNA viruses of the *Orthomyxoviridae* family. There are three major types: A, B and C. Influenza A viruses are further classified into subtypes - 16 HA and 9 NA - according to the nature of the viral envelope glycoprotein: hemagglutinin (H) or neuraminidase (N). Influenza A viruses stand out because they are able to infect both humans and animals, which characterizes influenza as a zoonotic disease. Also, their H and N antigens undergo considerable variations, unlike the same proteins in types B and C, which display minor alterations. Outbreaks of influenza type A occur in outbreaks in late fall and all over the winter season. Pandemics caused by influenza type A viruses frequently strike human populations^{1,12,19}.

They target different cell types across species - strains of human (H₁ and H₃) and equine (H₃ and H₁) viruses, for example, attach to receptors on tracheal epithelial cells, whereas avian influenza viruses attach to cells in the intestine^{23,25}.

Equine influenza A viruses (EIV) of subtype H₃N₈ have circulated among horses in Brazil since its isolation by Mc QUEEN *et al.*¹⁰, whereas subtype H₇N₇ viruses, isolated in 1976^{2,18}, are apparently

extinct or circulate at low levels in equine populations²⁴, which is consistent with recent observations by HEINEMANN *et al.*⁸, who reported serologic evidence of the H₇N₇ subtype of equine influenza A virus circulation in the north of Brazil. PENA *et al.*¹⁶ noted the presence of the equine H₃N₈ influenza A virus in 35.9% of 1,592 unvaccinated equines from farms situated in the south of the state of Pará-Brazil. DIELE *et al.*⁵ have reported the prevalence of antibodies against equine influenza (EIV) viruses among equines in Rio Grande do Sul (RS), Brazil: 986 sera samples from 1,506 equines (65.4%) revealed positivity for them. Recently, TONG *et al.*²¹ have isolated a new influenza A virus from little yellow-shouldered bats captured in two different locations in Guatemala and named it as H₁₇. This virus lineage differs in genetic constitution from all other known influenza A viruses, but it was not recognized as a new H subtype until now.

The acute respiratory disease caused by the equine influenza virus (EIV) features high morbidity and low mortality. The symptoms are: high temperature (± 38 °C), anorexia, coughing, dyspnea, nasal discharge and, depression. In some cases, it can escalate into a bacterial secondary infection (e.g. pneumonia), which may result in the death of the infected horses²². The disease also implies economic losses including turf race cancellations, reduced athletic performance in horses and treatment costs.

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Human parainfluenza viruses belong to the family *Paramyxoviridae* (*Paramyxovirus* genus), and are classified into four species: 1, 3 (*Respirovirus* genus), 2, 4a and 4b (*Rubulavirus* genus). Human parainfluenza 3 virus is a negative stranded RNA virus of the *Respirovirus* genus⁷.

They thrive worldwide, in temperate and tropical climates, all year round, through all seasons however, outbreaks of parainfluenza occur in late fall and early winter. Parainfluenza viruses present HN glycoproteins inserted in their envelopes, exhibiting hemagglutinin (H) and neuraminidase (N) features. In addition to infecting both humans and animals, influenza and parainfluenza viral infections share most of their symptoms. Some of the respiratory tract complications associated with the parainfluenza virus infection are: legionellosis, viral pneumonitis, pneumococcal pneumonia, melioidosis¹¹.

In the latter half of 1994, a new respiratory virus, a member of the *Parainfluenza* family, emerged in Australia and appears to be the cause of an infectious disease of zoonotic nature, which has been responsible for the deaths of humans and horses, two humans and 16 horses had died before 1998¹⁵. The authors suggested that the horses may have been infected from contact with infected birds and bats. The autopsy of these infected organisms revealed congested and hemorrhagic lungs, the microscopic lung examination revealing alveolitis with giant cells, and some syncytial formation. Sequenced analysis showed that PCR products from this virus isolated from the horses and one patient were identical. The phylogenetic analysis of the matrix protein segments indicated that this virus was related to other *Paramyxoviridae*. Fatal encephalitis has been reported in equines infected by a novel paramyxovirus¹⁴.

PHILBY *et al.*¹⁷ proposed menangle virus as a common name for an apparently new virus in the *Paramyxoviridae* family, isolated from stillborn piglets, in Australia. Sera from humans exposed to infected pigs and fruit bats also revealed seropositivity to these viruses. Additionally, CHANT *et al.*³ presented strong evidence of human infection by the same virus, transmitted to humans by infected pigs, but they were unable to explain the transmission mode.

FIELD *et al.*⁶ have reviewed a recently-identified zoonotic paramyxovirus, the Hendra virus which infects equines and then humans. They suggest minimizing the human contact with its natural host a species of fruit bats (*Pteropus* genus). Similarly, TAYLOR *et al.*²⁰ reported cases of human infection with Hendra virus through contact with equines infected by fruit bats (natural reservoir). Two patients were followed up after two and six years respectively and no evidence of virus shedding was found.

OBJECTIVE

This work was fueled by concerns about the circulation of influenza and parainfluenza viruses among equines. Therefore, it aimed to investigate antibody responses to both viruses in equine serum. This study's objective was to shed new light on the circulation of the influenza and parainfluenza viruses in animals in Brazil.

MATERIAL AND METHODS

Animals: Seventy-two equines from stud farms situated in Pirassununga (39), Águas de Lindóia (25) and Mairiporã (08), three

cities in the state of São Paulo, Brazil, were selected for blood serum analysis (Fig. 1).

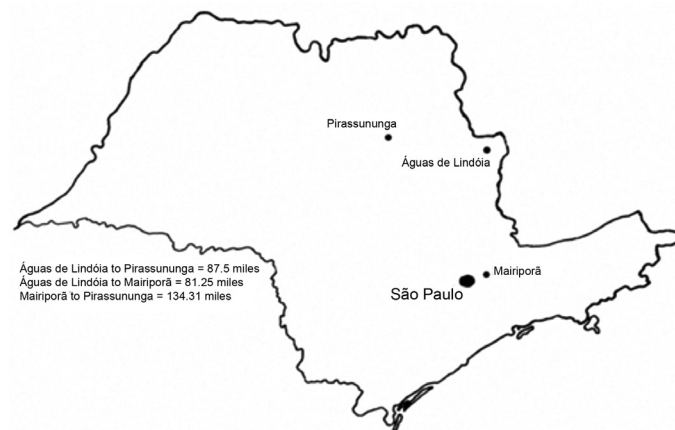


Fig. 1- Location of Pirassununga, Águas de Lindóia and Mairiporã cities in the state of São Paulo, Brazil.

The age, gender and breed of the equines from each city are described as follows:

- 1) Pirassununga city - 19 equines between five and 10 years old, 14 between 11 and 18 years old and six of an unknown age. Eleven were males and 28 females. As for the breed, 12 were Brazilian Turf, four were Pure Blood Arabian, six were Appaloosa, two were Pêga, five were Breton, one was Brazilian, one Schemule, one was Lusitano. Six of them were of an undefined breed.
- 2) Águas de Lindóia city - 18 equines between ≤ 2 and 10 years old, seven between 11 and 20 years old. Seventeen equines were males and eight females. Twelve animals were Mangalarga Paulista, nine were Mangalarga Mineiro; one was Appaloosa and one was Carapolina. Two of them were of an undefined breed.
- 3) Mairiporã city - five equines between five and nine years old, three over 10 years old. Seven were males and one was female. As for the breed, three were Arabian, one was Pure Blood English, one was Paint-equine, one was Appaloosa, one was Anglo-Arabian and one was Quarter Horse.

Serum: Blood serum samples were taken, heat-inactivated ($56^{\circ}\text{C}/30\text{ min}$) and then treated with Kaolin (20%) and erythrocytes (50%) according to MANCINI *et al.*⁹, DESHPANDE *et al.*⁴ and OIEN *et al.*¹³.

Serology: The hemagglutination inhibition (HI) test was performed according to MANCINI *et al.*⁹. Four HA units in 25 μL of viruses were used. Titers were expressed as hemagglutination inhibition units per 25 μL of sera (HIU/25 μL).

Antigens: Antigens of the influenza viruses types A/Eq1/SP/56 (A Eq1 H₇N₇) and A/Eq2/1/SP/85 (A Eq2 H₃N₈) and Parainfluenza 3 virus identified and provided by Adolfo Lutz Institute São Paulo, Brazil were used. These virus samples were grown in MDCK (Madin Darby canine kidney) cell cultures according to MANCINI *et al.*⁹.

Table 1
Antibody titers for influenza and parainfluenza viruses in equine sera

Virus	Samples	HIU/25µL							Total
		≤40	80	160	320	640	1280	2560	
PI	72	-(0)	2.80 (02)	16.66(12)	18.05(13)	30.55(22)	25.0(18)	6.94(05)	100(72)
H7N7	72	-(0)	20.83(15)	30.55(22)	35.72(25)	11.11(08)	1.38(01)	-(0)	98.6(71)
H3N8	72	-(0)	2.77 (02)	29.16(21)	44.44(32)	18.05(13)	2.77(02)	-(0)	97.22(70)

(HIU = Hemagglutination Inhibition Units).

RESULTS

The serology results obtained through the hemagglutinating inhibition (HI) test showed that 100, 98.6 and 97.22% of the equines responded with mean titers of ≥ 80 HIU/25µL to parainfluenza 3 and H₇N₇ and H₃N₈ subtypes of influenza A viruses, respectively (Table 1).

Figure 2 depicts the antibody responses to parainfluenza 3 virus in sera, in equines from Pirassununga and Águas de Lindóia and reveals a higher prevalence of the virus in equines from Lindóia city (HI = 1,324.48) than in the ones from Pirassununga city and from either of the age groups. The difference among mean titers was statistically significant (*p* = 0.0001). Mean HIU/25µL values for under nine-year-old and over 10-year-old equines, 917.89 and 738.06, respectively, showed no statistically significant difference (*p* > 0.05).

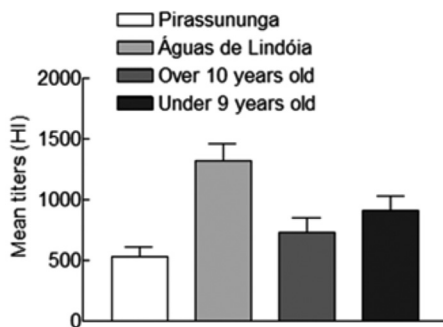


Fig. 2 - Antibody response to parainfluenza 3 virus in equines from Pirassununga and Águas de Lindóia cities and in two age groups. The results were expressed as means ± S.E.M.

Figure 3 depicts the mean titers of influenza A viruses (H₇N₇ and H₃N₈) for equines over ten years old (HIU/25µL of 266.35 and 381.93, respectively). Horses under nine years old showed mean titers (HIU/25µL) of 324.67 for influenza A(H₇N₇) and 353.68 for influenza A(H₃N₈). No statistically significant difference was observed.

Overall antibody responses to influenza A (H₇N₇) and A (H₃N₈) viruses and parainfluenza 3 virus were different. No statistically significant difference was observed between both influenza virus subtypes, however the mean titers for influenza and parainfluenza viruses were statistically different (*p* < 0.01) (Table 1).

DISCUSSION

Serum analysis confirmed the presence of influenza and parainfluenza

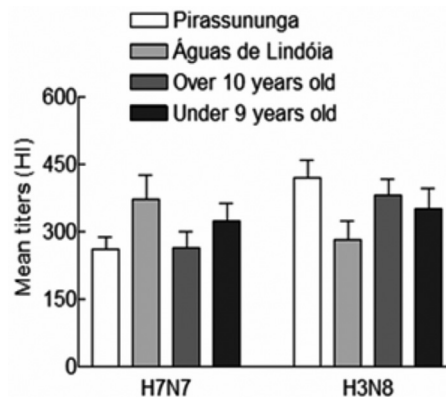


Fig. 3 - Antibody response to influenza A (H7N7) and A (H3N8) viruses in equines from Pirassununga and Águas de Lindóia cities and in two age groups. The results were expressed as means ± S.E.M.

type 3 viruses, in equines from Pirassununga, Águas de Lindóia and Mairiporã, three cities of the state of São Paulo State, Brazil. The distance between these cities is around ≥ 81.25 miles therefore, contact between the animals seems very unlikely.

Given that these equines had not been vaccinated against equine influenza or parainfluenza viruses, their antibody responses indicated a viral infection. They may have been infected from contact with infected birds, as previously suggested by PATERSON *et al.*¹⁵ (Table I).

Antibody responses, to equine influenza and parainfluenza viruses were not statistically different between the two age groups (equines under nine and over ten years old). Overall, all equines showed greater antibody response to parainfluenza 3 virus than to the equine influenza A viruses (H₇N₇ and H₃N₈).

Equines from Águas de Lindóia displayed higher antibody levels (HIU/25µL) against parainfluenza 3 virus than against equine influenza viruses, indicating no cross-reactivity among viruses. As for the influenza A viruses, (H₇N₇) subtype was more prevalent in the same city than the (H₃N₈) subtype, which, in turn, occurred more predominantly in Pirassununga.

The city of Mairiporã provided equines at the smallest sample size and lowest breed diversity. For this reason, it was not used in the comparison analysis of antibody responses.

Outbreaks of equine influenza in Brazil have been reported by

McQUEEN *et al.*¹⁰, PIEGAS *et al.*¹⁸, CUNHA *et al.*², PENA *et al.*¹⁶ and HEINEMANN *et al.*⁸, who recently observed the prevalence of equine influenza A(H₇N₇) virus in sera of horses from Uruará, State of Pará, Brazil. DIEL *et al.* 2005⁵ have also reported the prevalence of equine influenza in animals in the south of Brazil.

The zoonotic characteristics of influenza viruses can be attributed to their wide host range, from birds to mammals and even heterothermic animals, as participants in virus circulation^{1,4,9}. Fruit bats have also been regarded as a reservoir for both influenza and parainfluenza viruses^{6,14,17,20}.

Reports on the development of the parainfluenza 3 virus in equines are scarce, either nationally or internationally. In one relevant study from 1998, in Australia, PATERSON *et al.*¹⁵ reported the isolation of a new respiratory virus from horses and humans. Its characterization as a member of the *Paramyxovirus* genus was initially performed by virological tests and later confirmed by sequence analysis of the PCR products from the virus and the phylogenetic analysis of its matrix. The disease has been responsible for the deaths of human and horses, as observed in recent studies by TAYLOR *et al.*²⁰ and O'SULLIVAN *et al.*¹⁴, indicating its zoonotic nature. Equine influenza A(H₇N₇) and A(H₃N₈) viruses are currently common causes of disease in equines and have recently been reported to have infected cats and dogs^{1,4,25}. Considering that these viruses have adversely impacted economies all over the world, it is advisable to vaccinate equines.

In conclusion, our serology results confirmed the wide distribution of respiratory viruses, such as equine influenza and parainfluenza 3 virus, among equines in different cities of the state of São Paulo, Brazil. Because the animals had not been vaccinated against these viruses prior to our study, it is possible to conclude that the equines have been infected by them.

The presence of parainfluenza 3 virus is also evidenced by the fact that this virus is not equine-specific and that no cross-reactions were observed among the viruses studied here.

Further detailed studies of parainfluenza 3 virus infection in equines should be performed in order to develop preventive and protective measures for equine populations in Brazil.

RESUMO

Presença de vírus respiratórios em equinos do Brasil

Os equinos são susceptíveis aos vírus respiratórios, como o vírus influenza, e também tem sido citado o vírus parainfluenza. Doenças respiratórias têm impactado a economia em todo mundo. Este estudo intencionou determinar a presença dos vírus influenza e parainfluenza em equinos não vacinados de certas regiões do Estado de São Paulo, Brasil. Os soros coletados de 72 equinos, de diferentes cidades deste Estado, foram submetidos ao teste de Inibição da Hemaglutinação (IH) com objetivo de detectar anticorpos contra os referidos vírus, usando antígenos correspondentes. Cerca de 98,8% (72) e 97,2% (70) desses equinos responderam com títulos protetores (≥ 80 UIH/25 μ L) para os subtipos H₇N₇ e H₃N₈ de vírus influenza, respectivamente. Todos equinos (72) responderam com títulos protetores (≥ 80 UIH/25 μ L) contra o vírus parainfluenza 3. A diferença entre as médias de anticorpos contra

o vírus influenza A não foi estatisticamente significativa ($p > 0,05$). As médias de títulos dos vírus influenza e parainfluenza, por outro lado, demonstraram diferença estatisticamente significativa ($p < 0,001$). Esses resultados indicam melhor resposta de anticorpos pelos equinos ao vírus parainfluenza 3 do que ao vírus da influenza equina. Nenhuma diferença estatística foi observada nas respostas contra os vírus da influenza equina A (H₇N₇ e H₃N₈) e parainfluenza 3, com relação ao gênero (fêmeas e machos) e grupo etário (≤ 2 até 20 anos) nos equinos avaliados. Este estudo fornece evidência da presença concomitante dos dois subtipos vírus influenza A (H₇N₇ e H₃N₈) e do parainfluenza 3 em cavalos no Brasil. Portanto, é aconselhável a vacinação dos cavalos contra esses vírus respiratórios.

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