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/25µL) H, N, and H, N subtypes of influenza A viruses, respectively. All horses (72) also responded with protective titers (≥ 80 HIU/25µL) against the parainfluenza virus. The difference between mean antibody titers to H, N, and H, N 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 H, N, and H, N 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 (H, N, and H, N) 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 viruses frequently strike human populations, whereas subtype H among horses in Brazil since its isolation by Mc Queen et al. in 1976, which is consistent with recent observations by Heinemann et al. who reported serologic evidence of the H, N subtype of equine influenza A virus circulation in the north of Brazil. Pena et al. noted the presence of the equine H, N influenza A virus in 35.9% of 1,592 unvaccinated equines from farms situated in the south of the state of Pará-Brazil. DIEL et al. have reported the prevalence of antibodies against equine influenza (EIV) viruses among equines in Rio Grande do Sul (RS), Brazil: 99% of horses (≤ 2 to 20 years-old) sera samples from 1,506 equines (65.4%) revealed positivity for them. Recently, TONG et al. have isolated a new influenza A virus from little yellow-shouldered bats captured in two different locations in Guatemala and named it as H, N. 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.

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

Equine influenza A viruses (EIV) of subtype H, N, have circulated among horses in Brazil since its isolation by Mc Queen et al., whereas subtype H, N, viruses, isolated in 1976, are apparently extinct or circulate at low levels in equine populations, which has been reported by Heinemann et al. who noted the presence of the equine H, N influenza A virus in 35.9% of 1,592 unvaccinated equines from farms situated in the south of the state of Pará-Brazil. DIEL et al. 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. have isolated a new influenza A virus from little yellow-shouldered bats captured in two different locations in Guatemala and named it as H, N. 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 horses. 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 genus.

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, melioidosis.

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 1998. 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 paramyxovirus.

PHILBY et al. 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. 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. 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 (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).

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-équine, one was Appaloosa, one was Anglo-Arabian and one was Quarter Horse.

Serum: Blood serum samples were taken, heat-inactivated (56 °C/30 min) and then treated with Kaolin (20%) and erythrocytes (50%) according to MANCINI et al. Four HA units in 25 µL of viruses were used. Titters 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 H3N2) and A/Eq2/1/SP/85 (A Eq2 H1N1) 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.
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 ($p > 0.05$).

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

| Virus  | Samples | ≤40  | 80  | 160 | 320 | 640 | 1280 | 2560 | Total |
|--------|---------|------|-----|-----|-----|-----|------|------|-------|
| 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).

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Presença de vírus respiratórios em equinos do Brasil

Os equinos são susceptíveis aos vírus respiratórios, como o vírus influenza, e também têm 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 o vírus influenza, usando antígenos correspondentes. Cerca de 98,8% (72) responderam com títulos protetores (≥ 80 UIH/25µL) para o vírus influenza A, enquanto 97,2% (70) responderam com títulos protetores (≥ 80 UIH/25µL) para o vírus parainfluenza 3. Esta diferença entre as médias de anticorpos contra o vírus influenza A não foi estatisticamente significante (p > 0,05). As médias de títulos dos vírus influenza e parainfluenza, por outro lado, demonstraram diferença estatisticamente significante (p < 0,001). Esses resultados indicam melhor resposta de anticorpos pelos equinos ao vírus parainfluenza 3 do que ao vírus influenza equina. Nenhuma diferença estatística foi observada nas respostas contra os vírus da influenza equina A (H₃N₃) e H₃N₂) 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 (H₃N₃, e H₃N₂) e do parainfluenza 3 em cavalos no Brasil. Portanto, é aconselhável a vacinação dos cavalos contra esses vírus respiratórios.

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