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HUMAN ENTEROVIRUS INFECTION IN STRAY DOGS. SOME ASPECTS OF INTEREST TO PUBLIC HEALTH.

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SUMMARY

To investigate the possible role of domestic animals as reservoirs of human enteroviruses, we studied 212 stray dogs captured in different areas of the municipality of São Paulo. The captured animals were divided into 19 groups of 10 to 20 dogs each; faeces of 126 of the 212 dogs were processed for enterovirus isolation. The following viruses were isolated from 12 dogs: poliovirus type 1 (2 dogs), poliovirus type 3 (1 dog), echovirus type 7 (8 dogs) and echovirus type 15 (1 dog). Of the 12 infected animals, four had specific homotypic neutralizing antibody titres ≥ 16. All 212 animals were tested for the presence of neutralizing antibodies to human enteroviruses. The frequency of neutralizing antibodies present in titres of ≥ 16 was 10.3%, 3,8% and 4.3% for vaccinal prototypes of polioviruses 1, 2 and 3 respectively; 1,9%, 1.4% and 1.5% for wild prototypes of the same viruses, 11.3% for echovirus 7, and 2.4% for echovirus 15. The proportion of dogs with neutralizing antibodies varied with the virus studied. Some indication of the susceptibility of dogs to infection with human enteroviruses was demonstrated, and the importance of this fact for the Plan for Global Eradication of the Wild Poliovirus is discussed.

KEYWORDS: Enterovirus epidemiology; Poliovirus epidemiology; Enterovirus infection; Human enterovirus infection in animals; Poliovirus eradication.

INTRODUCTION

Enteroviruses infect human beings in all regions of the world, especially in areas of high population density and low socioeconomic status[2, 10]. Lower primates are known to be infected by enteroviruses, and some investigators have isolated enteroviruses and/or shown the presence of neutralizing antibodies against these agents in domestic animals, especially swine, cattle, dogs and cats, under natural and experimental conditions[1,5,9,11,13].

The global eradication of wild polioviruses implies, by definition, the extinction of these agents all over the world, a fact based on the assumption that the risk of reintroduction of these viruses in the human population is nonexistent permitting the interruption of all measures for the control of poliomyelitis at the world level, including the vaccination, as was the case for smallpox[8].

The good performance of the surveillance for infection caused by wild polioviruses is conditioned by a perfect knowledge of the mechanisms of perpetuation of polioviruses which are similar to those of all other enteroviruses. One of these mechanisms, i.e., the possible participation of domestic animals in the chain of transmission, has not been studied in depth.

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For this reason, the proposed Plan for Global Eradication of wild Polioviruses makes advisable to consider the possible importance of animals as reservoirs of human enteroviruses. A search for enterovirus infection in stray dogs captured in peripheral areas of the city of São Paulo, state of São Paulo, Brazil, is presented.

MATERIAL AND METHODS

The study was conducted on 212 stray dogs captured by the staff of the Municipal Center of Zoonoses Control in 13 peripheral neighborhoods of the city of São Paulo (SP). These neighborhoods are inhabited by a low-income population and their sewage service is poor if compared to the overall coverage for the city, that reaches about 60% of the residences. Dogs were captured in 1986, over a period of four months. A total of 19 capturing expeditions were performed, each resulting in 10 to 20 dogs, assigned to 19 groups studied (groups 1-19). The animals were sent to the kennel of the Municipal Center of Zoonoses Control where they were confined for 1-7 days, to collect blood and faecal samples. In some cases, animals were captured on consecutive days, so that two groups of animals were confined during the same period of time. As a consequence, on three occasions there was coincidence of the period of confinement, i.e., 6 of the 19 groups were confined during the same period of time and were in contact with each other.

Virus Isolation

Faecal samples were collected by rectal swab from 126 of the 212 studied dogs. Viruses were isolated using a continuous culture of human rhabdomyosarcoma embryonic (RD) cells. Samples were considered to be negative after three blind passages of 10 days each without appearance of the characteristic cytopathic effects. Positive cultures were frozen at -70°C and later identified and titrated by the technique of neutralization in cell culture, using a pool of specific sera against standardized L.B.M. enteroviruses and specific antisera for polioviruses 1, 2 and 3[5].

Determination of Neutralizing Antibodies

A single blood sample was obtained from each of 212 dogs. After serum inactivation at 56°C for 30 minutes, the presence of neutralizing antibody against enterovirus was detected according to GRANDIEN et al.[5] using human larynx carcinoma (Hep-2) cells. To improve the specificity of the assay, only the animals with titres ≥ 16 of specific neutralizing antibodies against the studied enteroviruses were considered as positive.

All the 212 dogs were studied for the presence of neutralizing antibodies against the following human enteroviruses: polioviruses of types 1, 2 and 3 of wild prototypes Mahoney (P1), MEF-1 (P2) and Saukett (P3) and vaccinal prototypes LS-c, 2 ab (P1), P712, Ch, 2 ab (P2) and Leon, $12a_1$ b (P3) and other enterovirus strains isolated from the animals under study.

RESULTS

Enterovirus Isolation

Human enteroviruses were isolated from 12 of the 126 studied stray dogs and identified as follows: poliovirus type 1 (two dogs), poliovirus type 3 (1 dog), echovirus type 7 (8 dogs), and echovirus type 15 (1 dog). One of the 8 dogs infected with echovirus type 7 (E_7) presented a homotypic neutralizing antibody titre of 16 and the other two, ≥ 64. One of the dogs infected with poliovirus type 1 (P_1) presented a homotypic neutralizing antibody titre of 16. In the remaining infected dogs, the titres of neutralizing antibodies for the respective isolated viruses were lower than 8 (Table 1).

Clusters of infected animals were detected by virus isolation or antibody detection with titre of ≥ 16. Four of the 8 dogs infected with E7 belonged to group 3 and two belonged to group 12. In both groups 20% of the dogs presented titres of neutralizing antibodies ≥ 16 against E7. In group 18, from which one P1 strain was isolated, 30% of the animals presented titres of homotypic neutralizing antibodies > 16 against the isolated virus.

Immune Status

The proportion of animals with ≥ 16 titres of specific neutralizing antibodies against the wild and vaccinal prototypes of polioviruses 1, 2 and 3 was 1.9%, 1.4%, 1.5% and 10.3%, 3.8% and 4.3%, respectively (Table 2). The distribution of animals with titres ≥ 16 of neutralizing antibodies against wild poliovirus strains was sporadic, with only 4 of the 19 groups showing these titres and never with more than one positive dog per group. The distribution of the same antibodies against polioviruses of vaccinal strains was more homogeneous, with these antibodies being present in 17 of the 19 groups studied. In three of these 17 groups the proportion of animals with titres of antibodies ≥ 16 reaches higher values; 15% in two groups and 30% in the third.

The frequency of animals with titres ≥ 16 of neutralizing antibodies against the isolated strains of P1, P3, E7 and E15 was 1.5%, 4.3% 11.3% and 2.4%, respectively (Table 3). Of the nine animals with titres ≥ 16 of neutralizing antibodies to the isolated P3 strain, four had identical titres for the vaccinal strain of the same type and only one for the wild strain. Of the three animals with titres ≥ 16 for the isolated P1,

TABLE 1

Distribution of isolated viruses and the respective titre of homotypic neutralizing antibodies in infected dogs, and the proportion of immune animals in the corresponding group

Group	Dog Nr.	Isolated virus	Neutralizing antibody titre	Proportion of immune animals in the group**
1	14	P_3	<8	5%
2*	23	E_{15}	<8	0%
2*	25	E_7	<8	0%
3*	32	E_7	<8	20%
3*	35	E_7	>64	20%
3*	36	E_7	8	20%
4	42	E_7	16	15%
10	109	P_1	<8	10% ***
12	125	E_7	<8	20%
12	126	E_7	>64	20%
16	161	E_7	<8	0%
18	185	P_1	16	30%

* These animals were confined during the same period of time.

** Proportion of dogs with titres of > 16 of homotypic neutralizing antibodies to the isolated virus in same group.

*** Neutralizing antibodies against P_1 of a vaccinal strain.

1 showed identical titres for the vaccinal strain of the same virus.

The 24/212 animals (11.3%) with titres ≥ 16 of neutralizing antibodies against E_7 belonged to eight of the 19 groups studied, at proportions ranging from 10.0% to 40.0%, whereas the five animals with the same antibody levels against E_{15} belonged to five groups, and only two of these five dogs were confined during the same period of time.

None of the 44/61 (72.1%) animals presenting neutralizing antibody titres ≥ 16 to any of the enteroviruses studied had antibodies to more than one of the viruses studied.

TABLE 3

Neutralizing antibodies to isolated strains* of P_1 and P_3, E_7 and E_{15}, in 212 stray dogs captured in peripheral areas of the city of São Paulo, SP, Brazil.

	Poliovirus* serotype		Echovirus* serotype	
	1	3	7	15
Titre	Nr. (%)	Nr. (%)	Nr. (%)	Nr. (%)
< 8	209 (98.6)	197 (92.9)	156 (73.6)	193 (91.0)
8	- (-)	6 (2.8)	32 (15.1)	14 (6.6)
16	1 (0.5)	3 (1.4)	10 (4.7)	3 (1.4)
32	1 (0.5)	1 (0.5)	11 (5.2)	2 (0.9)
64	1 (0.5)	5 (2.4)	3 (1.4)	- (-)
Total	212 (100)	212 (100)	212 (100)	212 (100)

* Strains of poliovirus type 1 and 3 (P_1 and P_3) and echovirus types 7 and 15 (E_7 and E_{15}) isolated from faeces of the dogs studied.

DISCUSSION

The present results are similar to those reported by other investigators[5,9,11]. The frequency of infection by human enterovirus verified in stray dogs from peripheral areas of the city of São Paulo is probably related to the inadequate sewage service in these neighborhoods. In these areas the sewage is dumped on the street and frequently becomes a source of drinking water for these animals.

The hypothesis that these animals are susceptible to infection by human enteroviruses is supported by the fact that four of the 12 infected dogs had titres ≥ 16 of specific neutralizing antibodies to the viruses isolated from faeces. The lack of homotypic neutralization anti-

TABLE 2

Neutralizing antibodies to wild and vaccinal prototypes of polioviruses 1, 2 and 3, in 212 stray dogs captured in peripheral areas of the city of São Paulo, SP, Brazil

	"Wild" polioviruses Serotype			Vaccinal polioviruses Serotype		
	1	2	3	1	2	3
Titre	Nr. (%)	Nr. (%)	Nr. (%)	Nr. (%)	Nr. (%)	Nr. (%)
< 8	205 (96.7)	207 (97.7)	200 (94.3)	178 (84.0)	196 (92.5)	188 (88.7)
8	3 (1.4)	2 (0.9)	9 (4.2)	12 (5.7)	8 (3.7)	15 (7.0)
16	3 (1.4)	2 (0.9)	1 (0.5)	12 (5.7)	5 (2.4)	3 (1.4)
32	- (-)	- (-)	1 (0.5)	3 (1.4)	2 (1.4)	1 (0.5)
> 64	1 (0.5)	1 (0.5)	1 (0.5)	7 (3.2)	1 (0.5)	5 (2.4)
Total	212 (100)	212 (100)	212 (100)	212 (100)	212 (100)	212 (100)

bodies to the viruses isolated from 8 of 12 infected dogs may be attributed to the following causes: i) these viruses may be simply passing through the gut of the animal without detectable antibodies, ii) the animals had been confined for no longer than 7 days and there was no sufficient time for the development of specific antibodies, and iii) the animals had a delayed immune response to enterovirus infection, as also observed in man.

After experimentally infecting six dogs by oral administration of E_6, PINDAK & CLAPPER[12] were able to isolate the same virus from the faeces of four animals and from the blood of one of them, and none responded with the formation of homotypic neutralizing antibodies up to 35 days after the experiment. However, when these same dogs were later inoculated with live E_6 by the intramuscular route, they responded to the stimulus with high titres of neutralizing antibodies.

Using the criteria proposed by KLEIN[6] and by GREW et al.[5] for the determination of the specificity of neutralizing antibodies to human enteroviruses in mammals, the following facts favour the hypothesis that the neutralization antibodies found were specific: i) stability of the neutralizing substance at 56°C for 30 minutes; ii) in 44/61 (72.1%) of the animals presenting titres of neutralizing antibodies ≥ 16 against any of the enteroviruses studied, these antibody levels were found against only one of the viruses studied; iii) of the nine animals that presented titres of neutralizing antibodies ≥ 16 for the isolated P_3, four showed identical titres for the vaccinal strain of the same virus and one for the wild strain. Furthermore, the presence of neutralizing antibodies to prototypes of wild poliovirus strains was infrequent and there were no clusters of dogs with this neutralizing antibodies in the same group. These results agree with the absence of reports of poliomyelitis and/or circulation of the wild poliovirus in the city of São Paulo for some time preceding the present study.

The data become more consistent if we compare the results of the serologic tests carried out with vaccinal prototypes of poliovirus, which revealed a high proportion of dogs with neutralizing antibodies against the vaccinal prototypes of P_1, P_2 and P_3, a fact compatible with the wide circulation of these viruses in the environment, at least for 2 to 3 months, after the two yearly "National Days of Vaccination against Poliomyelitis" in Brazil. Also the high frequency of animals with neutralizing antibodies to E_7, corresponding to the virus most frequently isolated from the dogs, should be mentioned, as well as the concentration of animals with high titres within few groups of dogs, in many cases coinciding with the isolation of E_7 virus.

The present results are of interest in terms of regional eradication of polioviruses in the Americas since, by definition, regional eradication or elimination of an agent implies the risk of its reintroduction[8]. Thus, in this case it is imperative to maintain all control measures and especially to improve surveillance which, in addition to routine activities, should involve, for example, the identification of residues of infectious source, even if this kind of infectious source would have fewer repercution on the incidence of poliomyelitis.

The behavior of polioviruses presents certain characteristics that emphasize the need for the detection of the sources of infection of minor epidemiologic importance. Among these characteristics are: i) their ability to circulate silently in the human population and to reinfect immune individuals; and ii) the protection through herd immunity is limited since individuals fully vaccinated may be involved in the chain of transmission[7,14].

The reintroduction of wild poliovirus 3 in Canada in 1993 due to an epidemic in Holland[2] illustrates the importance of surveillance for the early detection of similar situations and the need for a better understanding of points still awaiting full clarification with respect to the mechanisms of poliovirus perpetuation.

According to the present results, the existence of thousands of stray dogs in large Brazilian cities and also possibly in the largest cities of the Third World, living in intimate contact with the human population, mainly children, let us consider the possible importance of these animals as residual source of infection.

However, some questions about the real importance of domestic animals in the mechanisms of perpetuation of enteroviruses, especially polioviruses, still await conclusive answers. The most important one is the possibility that stray dogs, when present at high population densities, permit the maintenance of the circulation of wild polioviruses even without human participation as a reservoir.

RESUMO

Infecção por enterovirus humanos em cães errantes. Alguns aspectos de interesse em Saúde Pública.

Foram estudados 212 cães errantes, capturados em áreas periféricas do município de São Paulo, com o objetivo de investigar o possível papel desses animais como reservatórios de enterovírus humanos. Os animais capturados foram divididos em 19 grupos compostos por 10 a 20 animais cada um. Foram efetuadas tentativas de isolamento em amostras de fezes em 126/212 cães,

obtendo-se resultados positivos em 12 deles, assim distribuídos: poliovirus tipo 1 (dois cães), poliovírus tipo 3 (um cão), echovirus tipo 7 (oito cães) e echovirus tipo 15 (um cão). Dos 12 animais infectados, quatro apresentaram anticorpos neutralizantes homotípicos com títulos ≥ 16. Todos os 212 cães foram submetidos a pesquisa de anticorpos neutralizantes contra enterovírus humanos. As frequências de anticorpos neutralizantes com títulos ≥ 16 foram de 10,3%, 3,8% e 4,3%, respectivamente, contra protótipos vacinais de poliovírus 1, 2 e 3 e 1,9% 3,8% e 4,3% contra protótipos "selvagens" dos mesmos vírus; de 11,3% contra echovírus tipo 7 e de 2,4% contra echovírus tipo 15. São discutidos dados sugestivos da suscetibilidade de cães à infecção por enterovírus humanos e a possível importância desse fato para o Plano de Erradição do Poliovírus "Selvagem".

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