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EXPERIMENTAL INFECTION OF HORSES WITH A-EQUI 2/MIAMI/1/63/AND HUMAN A2/HONG KONG/1/68 INFLUENZA VIRUSES. II. ANTIBODY RESPONSE TO THE INFECTION

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Summary. — Experimental intranasal infection of four horses with A-equi 2/Miami/1/63 influenza virus revealed haemagglutination-inhibiting (HI) antibodies against heterologous A-equi 1/Praha/56 influenza virus (recall phenomenon), but no antibody against the infecting virus when the potassium periodate-treated sera were tested in dilutions higher than 1 : 16. A booster dose with the infecting virus stimulated the production of homologous antibody and antibody against the heterologous human A2/Hong Kong/1/68 influenza virus [both inhibitor-sensitive (IS) and inhibitor-resistant (IR) variants]. Further challenge of these horses with A2 Hong Kong influenza virus led to some increase in antibody titre against both the challenging virus and A-equi 1 and A-equi 2 viruses.

Intramuscular (im) administration of A-equi 2 virus to two horses did not reveal the recall phenomenon to A-equi 1 virus, nor did it induce homologous antibodies. A booster dose of A-equi 2 virus given 50 days after infection (p.i.) stimulated the animals to produce antibody to the homologous virus (titres 64—512) and to A2/Hong Kong/1/68 virus (both IS and IR variants). Later challenge of animals with A2 Hong Kong virus did not significantly change the antibody titres earlier achieved.

Intranasal infection with the IS variant of A2/Hong Kong/1/68 virus stimulated 3 out of 4 horses to produce antibodies against the homologous virus variant. After challenge with A-equi 2 virus, antibodies against heterologous A2 Hong Kong virus (both IS and IR v ariants) reached high levels (64—256). No antibody to the challenging virus was detectable in serum dilutions of 1 : $\leq$16. No antigenic relationship of A-equi 1, A-equi 2 and A2 Hong Kong influenza viruses with A-duck BV/63 influenza virus was detected in serum dilutions of 1 : 16 and higher.

It is suggested that the virions of A-equi 1, A-equi 2 and human A2 Hong Kong influenza viruses share some common antigens.

Introduction

Clinical features of infection of, and virus recovery from, horses infected with either A-equi 2/Miami/1/63 or human A2/Hong Kong/1/68 influenza viruses were described in the previous paper (Blaškovič *et al.*, 1969). This is to describe the immunological response of the infected animals to the viruses administered as revealed by the HI test. Data on the antibody response in some selected horses as observed in the gel double diffusion reaction will be reported later (Styk *et al.*, 1970).

Materials and Methods

Experimental animals and mode of infection. Horses of different age groups and both sexes were infected as described in the previous paper (Blaškovič *et al.*, 1969). Blood samples were taken at intervals mentioned along with the results.

Viruses. Infectious allantoic fluids harvested after 3 days of incubation with A-equi 1/Praha/1/56, A-equi 2/Miami/1/63, human A2/Hong/Kong/1/68 (both IS and IR variants) and A-duck BV/63 influenza viruses were used in HI tests throughout. The passage history of the strains and their infectious and haemagglutinating titres were described previously (Blaškovič *et al.*, 1969).

Immunization of rabbits and mice with A-equi 2/Miami/1/63 and human A2/Hong Kong/1/68 influenza viruses. For confirmation and interpretation of serological results obtained after infection of horses, hyperimmune sera to the respective viruses were produced in rabbits and mice.

Rabbits were immunized with either A-equi 2/Miami/1/63 or human A2/Hong Kong/1/68 influenza viruses by two 5-ml doses of the respective infectious allantoic fluid given intraperitoneally at a 10 day interval. Two further doses, 10 days apart, of 5 ml infectious allantoic fluid were given im with adjuvant (arlacel and paraffin oil). Twelve days after the last dose, the animals were bled and the serum tested for HI activity. From a part of the serum obtained a conjugate with fluorescein isothiocyanate was prepared.

Mice were immunized intranasally in light ether anaesthesia with the respective infectious allantoic fluid. A second dose of 0.2 ml infectious allantoic fluid was given intraperitoneally 14 days apart. Fourteen days later, the mice were bled and the serum used for HI tests and preparation of conjugate for immunofluorescence (Blaškovič *et al.*, 1964).

Serology. HI tests were performed by the procedure described (Blaškovič *et al.*, 1969).

Results

Immunological response of horses intranasally infected with A-equi 2/Miami/1/63 virus

The results obtained were based on titres of 16 and higher, because the initial serum dilution, due to potassium periodate treatment, was 1 : 8. Blood samples were taken on day 14 and 34 p.i. On day 50 p.i., the animals were boostered im with 10 ml of infectious allantoic fluid containing A-equi 2 virus. Blood samples were then taken on days 57, 64 and 85. On day 90 p.i., 10 ml of infectious allantoic fluid containing A2 Hong Kong influenza virus was administered intravenously (iv). Further blood samples were taken on days 100, 110, 120 after primary infection with A-equi 2 influenza virus. HI tests were performed with A-equi 1, A-equi 2, A2 Hong Kong (IS and IR variants), and A-duck BV/63 influenza viruses.

The results presented in Fig. 1 can be summarized as follows:

a) The intranasal infection with A-equi 2 influenza virus was not accompanied by detectable antibody formation against homologous virus, when

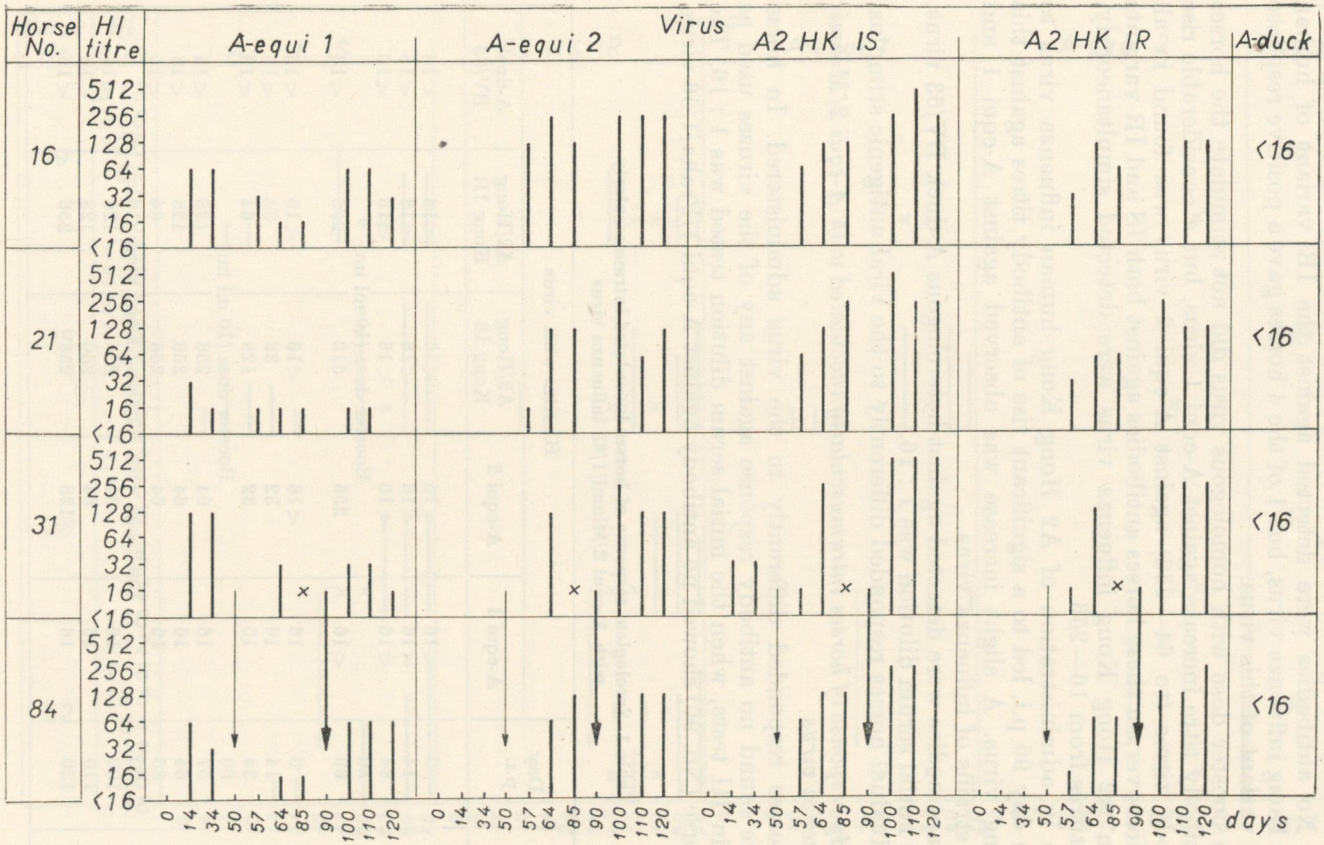

Fig. 1.

HI antibody response of horses infected with A-equi 2/Miami/1/63 influenza virus and later challenged with A2/Hong Kong/1/68 (A2 HK) human influenza virus

◀—— Booster dose (im) ◀—— Challenge with A2/Hong Kong/1/68 virus (iv) × Not done

the initial serum dilution tested was 1 : 16, though all 4 horses replied with antibody formation against A-equi 1 influenza virus in titres ranging from 32—128. No antibodies were detected against the IR variant of human A2 Hong Kong influenza virus, but 1 of the 4 horses gave a positive response to the IS variant of this virus.

b) The booster dose with homologous virus did not stimulate the horses to an antibody titre increase against A-equi 1 virus, but a considerable rise in antibody titres (to 64—256) against A-equi 2 virus was found in all horses. Moreover, in these horses antibodies against both IS and IR variants of human A2 Hong Kong influenza virus were detected simultaneously, reaching titres from 16—256.

c) The iv administration of A2 Hong Kong human influenza virus to horses on day 90 p.i. led to a significant rise of antibody titres against this challenging virus. A slight increase was observed against A-equi 1 and A-equi 2 strains of influenza virus.

d) No antibodies were detected against heterologous A-duck BV/63 virus, when the initial serum dilution was 1 : 16.

e) Individual horses responded differently to the viral antigenic stimulus.

Antibody response of horses intramuscularly inoculated with A-equi 2/Miami/1/63 influenza virus

Two horses responded differently to the virus administered. In horse No. 17 we found no antibody response against any of the viruses used as antigens in HI tests, when the initial serum dilution tested was 1 : 16. The second horse (No. 90) showed no antibody against A-equi 1/Praha/1/56 virus,

Table 1. Serological response of horses inoculated intramuscularly with A-equi 2/Miami/1/63 influenza virus

Horse No.	Day p.i.	HI titre with virus				
		A-equi 1	A-equi 2	A2/Hong Kong IS	A2/Hong Kong IR	A-duck BV/63
17	0	<16	<16	<16	<16	<16
	14	<16	<16	<16	<16	<16
	34	<16	<16	<16	<16	<16
	50	Booster dose (10 ml im)				
	69	<16	256	512	256	<16
90	0	16	<16	<16	<16	<16
	14	16	32	32	32	<16
	34	16	32	128	64	<16
	50	Booster dose (10 ml im)				
	57	16	64	256	128	<16
	64	16	64	256	128	<16
	85	16	64	256	64	<16
	90	Challenge with A2/Hong Kong virus (10 ml iv)				
	100	16	64	256	128	<16
	110	16	64	256	128	<16
	120	16	128	256	256	<16

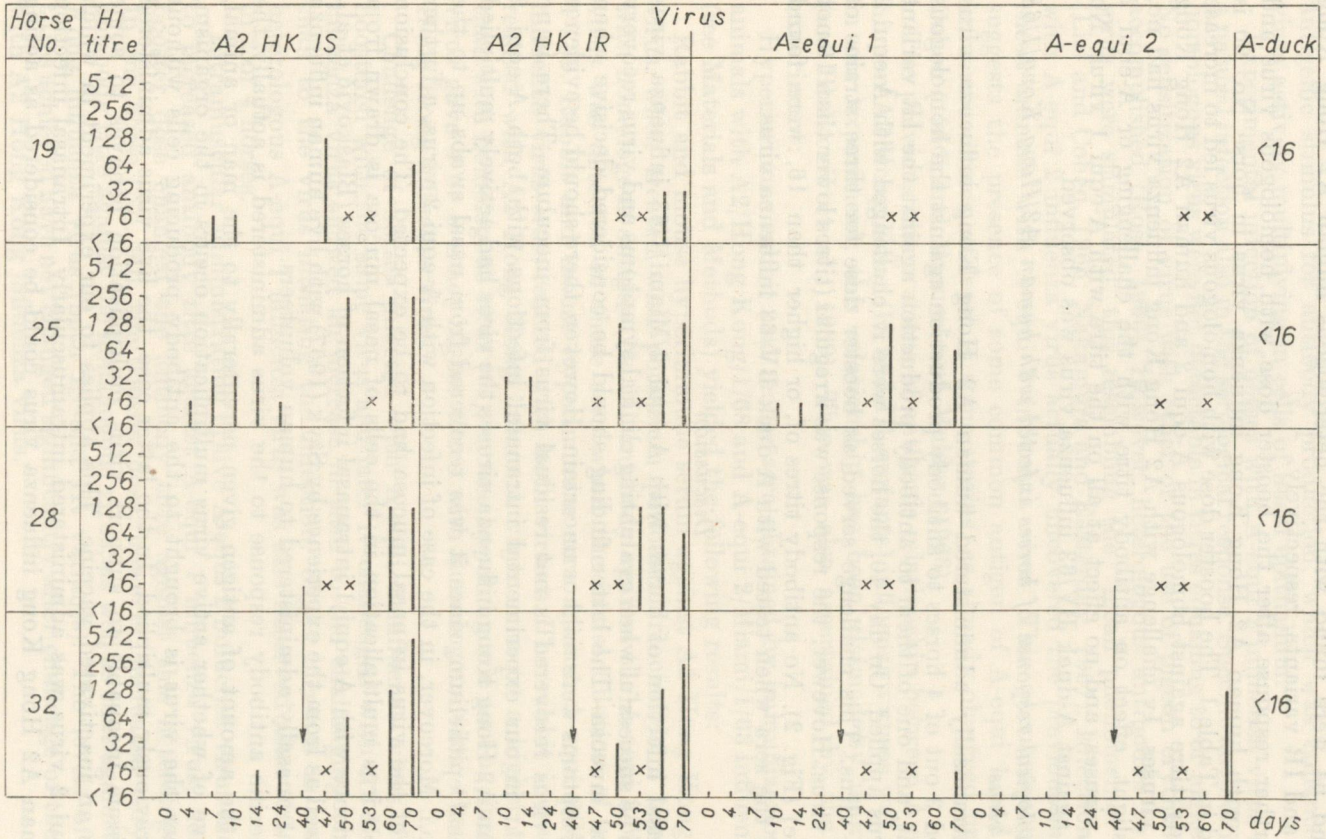

Fig. 2.

HI antibody response of horses infected with A2/Hong Kong/1/68 (A2 HK) human influenza virus and later challenged with A-equi 2/Miami/1/63 virus

◀—— Challenge with A-equi 2 virus (iv) × Not done

but a titre of 32 was noted with the homologous A-equi 2 virus and titres of 128 and 64 were found with the heterologous human A2 Hong Kong virus, IS and IR variants, respectively.

The further response after the booster dose with homologous virus and challenge with human A2 Hong Kong influenza virus in horse No. 90 is illustrated in Table 1. The booster dose with homologous virus led to increase in antibody titre against homologous A-equi 2 and human A2 Hong Kong influenza viruses. Iv challenge with A2 Hong Kong influenza virus had no, or only slight, effect on antibody titre with the challenging or A-equi 2 influenza viruses, and no effect at all on the titre with A-equi 1 virus. No antibody against A-duck BV/63 influenza virus was observed.

Immunological response of horses infected with human A2/Hong Kong/1/68 influenza virus

The intranasally administered human A2 Hong Kong influenza virus stimulated 3 out of 4 horses to antibody production against the homologous IS variant, and one of them to antibody production against the IR variant of the virus applied. On day 40, the horses were iv challenged with A-equi 2 influenza virus. This challenge served as booster dose for three strains of influenza virus. Hovewer, the response was irregular (titres lower than 16 not detectable) (Fig. 2). No antibody titres of, or higher than 16, were found in any of the sera when tested with A-duck BV/63 influenza virus.

Discussion

Intranasal infection of horses with A-equi 2/Miami/1/63 influenza virus proved to be successful when evaluating clinical symptoms and virus recovery from nasal mucosa. The latter finding should be considered decisive when clinical symptoms were mild or uncertain. However, there should be evidence that the virus recovered is not residual virus from inoculum. There is no doubt that in our experimental intranasal infections with both A-equi 2 and human A2 Hong Kong influenza viruses the virus had actively multiplied in the nasal epithelium, since it was recovered from nasal swabs up to the 5th day p.i. Moreover, in the case of infection with A-equi 2 virus, a further presence of the virus in nasal mucosa had to be expected. The conclusion on active virus multiplication in the cells of nasal mucosa is drawn from our experience with A-equi 1 intranasal infection of horses (Blaškovič et al., 1966) as well as from the experience by Styk (1957) with live human influenza viruses intranasally administered to human volunteers.

The specific antibody response to the virus administered is actually the result of the amount of antigen given parenterally to the man or animal, irrespective of whether active virus multiplication occurs in the organism, or whether the virus is brought to the antibody producing cells without having previously multiplied. In the latter case, the live virus administered to the tissue incapable to support virus multiplication behaves to some extent as an inactivated vaccine. This applies to our experiment in which live A-equi 2 virus was administered intramuscularly. Intranasal infection with human A2 Hong Kong influenza virus could be considered as acting

in both ways. The amount of virus administered could serve as satisfactory antigenic stimulus for antibody production, which was enhanced by the virus actively multiplying in cells of nasal epithelium.

Experimental infection of horses with A-equi 2 influenza virus resembled a natural infection also in the serological response. Specific antibodies against homologous virus were not detectable in titres equalling to or higher than 16. No sensitization of virus with ether or the use of pigeon erythrocytes was accomplished (Rose, 1966). However, heterologous antibodies to A-equi 1 virus were stimulated, this resembling the "recall phenomenon" reported (Lief and Cohen, 1966; Nakamura and Easterday, 1967). Infection of horses with A-equi 2 influenza virus actually uncovered the previous infection of these animals with A-equi 1 influenza virus. This phenomenon strongly suggests the presence of some common antigen of A-equi 1 and A-equi 2 influenza viruses, at least in some strains, as already observed by Lief and Cohen (1966) and Masurel and Mulder (1966).

The previous observations of antigenic relationship between A-equi 2 and human A2 Hong Kong (IS and IR variants) influenza viruses was confirmed in our experiment; this relationship has been claimed by many authors (see Blaškovič et al., 1969). This was also ascertained by the following experiment:

Hyperimmune rabbit and mouse sera obtained by immunization of these animals with A2/Hong Kong/1/68 and A-equi 2/Miami/1/63 influenza viruses (see Materials and Methods) yielded the following results:

Rabbit and mouse hyperimmune serum against A2 Hong Kong influenza virus, when treated with potassium periodate, reacted in a titre of 2048 with homologous antigen. No HI antibody against the A-equi 2/Miami/1/63 virus was detected in these sera when the initial dilution of serum was 1 : 32. Rabbit and mouse hyperimmune sera against A-equi 2/Miami/1/63 influenza virus cross-reacted with both viruses. The rabbit immune serum reacted with A2 Hong Kong influenza virus even to a higher titre (128) than with homologous virus (64); mouse hyperimmune sera against A-equi 2 virus gave 4 or 8 times higher titres with homologous virus than with A2 Hong Kong influenza virus.

Challenge of horses infected with A-equi 2 influenza virus, further im boostered with the same virus and later iv challenged with A2 Hong Kong influenza virus showed an increase in titre to the challenging virus rather than to the virus initiating the infection. Slight increase in antibody to heterologous A-equi 1 influenza virus was also observed after this challenge in 2 of 4 horses.

Intramuscular administration of live A-equi 2 influenza virus did not lead to increased antibody titre against A-equi 1 influenza virus, nor had the booster dose changed the antibody titre against this heterologous virus. In one horse (No. 90), the booster dose, however, increased very soon the antibody titre against the homologous virus, as well as that against A2 Hong Kong influenza virus (both IS and IR variants). The levels of antibody titres achieved have not changed significantly after challenge of the organism with A2 Hong Kong influenza virus.

Horses infected with A2 Hong Kong influenza virus replied in the majority (3 out of 4) of cases with antibody production against the homologous IS variant of the virus in titres equal to or higher than 16. Only one horse developed antibodies against the IR variant of this virus and showed the "recall phenomenon" to A-equi 1 influenza virus. This infection was inert to antibody production to A-equi 2 influenza virus. Challenge with A-equi 2 influenza virus stimulated the horses to antibody increase against heterologous virus (A2 Hong Kong human influenza virus, both IS and IR variants) and failed to induce homologous antibodies in detectable amounts, except in horse No 32 (30 days after challenge).

A-equi 2 influenza virus, iv administered to horses, increased the level of preexisting heterologous antibodies to a higher degree than the homologous ones. This is suggested by the results obtained with our hyperimmune rabbit and mouse sera to A-equi 2 or A2 Hong Kong influenza viruses, and was well documented by Masurel (1968) vaccinating man with A-equi 2 influenza virus.

No cross-reaction of either A-equi 2 and A2 Hong Kong influenza viruses with A-duck BV/63 influenza virus was detected when the initial serum dilution was 1 : 16.

A-equi 1/Praha/1/56, A-equi 2/Miami/1/63 and human A2/Hong Kong/1/68 influenza viruses share some common antigens, the quantitative distribution of which in the appropriate virions is difficult to interpret from the present experiments.

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