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Susceptibility of a yak to influenza A viruses and presence of H3N2 antibodies in animals in Nepal and India*

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Naturally acquired antibody to H3N2 human influenza antigens was found in a yak–zebu crossbred in Nepal. Serial inoculation of a yak, negative for antibody, produced a response to A/Hong Kong/1/68 (H3N2), A/England/42/72(H3N2), and A/Prague/1/56 (Heq1 Neq1) influenzavirus strains. Single radial diffusion tests showed that cattle and goats in West Bengal, India, and water buffaloes and cattle in Kathmandu, Nepal, also had antibodies against the H3N2 antigens. Haemagglutination-inhibition antibodies to equine influenzaviruses were not found in human, goat, cattle, chicken, and dog sera, nor were antibodies to avian viruses found in human or equine serum.

Yaks (*Bos grunniens*) live in close association with man in the high Himalayan mountains. When they are bred to zebu bulls, the resulting crossbreds thrive at lower altitudes than yaks and provide a primary source of milk. Antibody against the H3N2 antigens of recent human influenzavirus strains was found in one crossbred living in Kathmandu zoo. This animal was thus a host for "human strains" of influenzavirus and was a potential host for new pandemic strains, which could arise by genetic recombination of animal and human influenzaviruses (9, 11).

In order to carry out serological studies on the susceptibility of yaks to influenza, a 1-year-old male yak was transported by air in February 1972 from a village at 4 000 m elevation to Kathmandu (1 340 m).

MATERIALS AND METHODS

Intact X-31 strain of A/Hong Kong/1/68 (H3N2) influenzavirus in gels [a] (17) was used in single radial diffusion (RD) tests in order to detect antibodies to the haemagglutinin and neuraminidase but not to matrix protein or ribonucleoprotein antigens. Haemagglutination inhibition (HI) tests were also carried out to detect antibodies. Viruses [a] for inoculation and for the HI tests were grown and titrated in eggs. Eight units of ether-treated antigen, type O human erythrocytes, and sera treated with receptor-destroying enzyme (RDE) or heat-trypsin-KIO_4 (12) were used in the HI test.

Animal sera were collected during the periods indicated in Table 1. Human sera were collected from blood donors, maternity patients, and children in Kathmandu during 1972–73 (19).

RESULTS

At a high altitude, exercising the yak during unusually warm ambient temperatures resulted in increases in its rectal temperature from 38.6°C to 40.7°C, pulse from 55 to 90 beats per min, and respirations from 20 to 88 per min. Similar changes were observed in Kathmandu; measurements of these factors are therefore not always reliable as indicators of infection.

The administration of 3×10^3 egg infectious units (EIU) of A/Hong Kong/1/68 (H3N2), 2×10^6 EIU of A/England/42/72 (H3N2), and 1×10^5 EIU of A/Prague/1/56 (Heq1Neq1) influenzaviruses was carried out intranasally and intratracheally to the yak over a period of 81 days, as indicated in Fig. 1.

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[a] Kindly supplied by the World Influenza Centre, London, England.

Table 1. Results of single radial diffusion tests with X-31 strain of A/Hong Kong/1/68 (H3N2) influenzavirus on animal sera from Nepal and India

	Nepal [a]		India [b]	
	Veterinary lab.	Khumaltar farm	Singur	Ichag
cattle	5/6 [c]	1/3	6/6 [d]	4/13
goats	0/28	0/15	5/14	0/6
water buffaloes	1/1	1/1		
dogs			0/4	0/2
chickens		0/1	0/1	0/2
pigs		0/10		
sheep				0/2
horses [a,b]	0/19		0/2	
yaks	1/3 [e]			

[a] In Nepal, the animals were located at the Veterinary Laboratory, Kathmandu, and at Khumaltar farm which is 5 km away. The horses were stabled in Kathmandu.

[b] In India, the animals were located in Singur, 40 km from Calcutta, and Ichag, a village near the West Bengal–Bihar border. The horses were stabled in Calcutta.

[c] Number positive/total tested.

[d] Seroconversion occurred between 7 June 1972 and 29 July 1972 in a 6-month-old calf. The sera from India were drawn between 7 June 1972 and 25 September 1972, and from Nepal between 1 January 1973 and 28 August 1973.

[e] The positive animal was a yak–zebu crossbred living in Kathmandu zoo and the two negatives were yaks from Lang Tang village (elevation 4000 m).

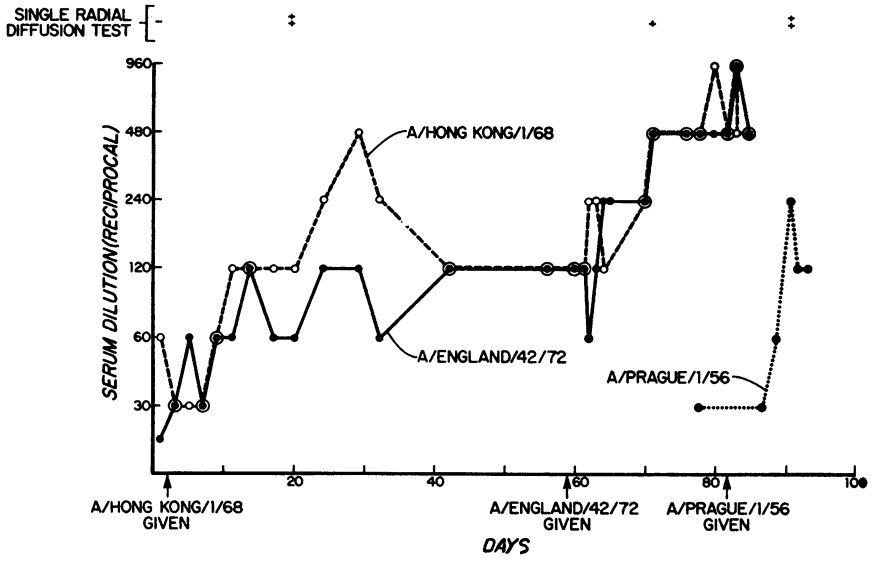

Fig. 1. Results of haemagglutination inhibition and single radial diffusion tests on sera from a yak after inoculation with A/Hong Kong/1/68 (H3N2), A/England/42/72 (H3N2), and A/equine/Prague/1/56 (Heq1Neq1) influenzaviruses.

HI tests on the sera treated with RDE showed that inhibitors were present at dilutions of up to 1/60 for A/Hong Kong/1/68 and A/England/42/72, and 1/30 for A/Prague/1/56 (Fig. 1). The titres of the inhibitors in sera treated only with heat (56°C for 30 min) were 1/240 for A/England/42/72. In contrast, sera treated with heat-trypsin-KIO_4 contained no inhibitory activity. However, the antibody titres to both the H3N2 antigens were reduced 4 to 8-fold. The RD test was not affected by these inhibitors (Fig. 1).

The primary antibody response to infection with A/Hong Kong/1/68 influenzavirus occurred within 9 days post inoculation (p.i.) while mild respiratory symptoms, including coughing, and malaise were observed on the 6th day (Fig. 1). Since A/Hong Kong/1/68 and A/England/42/72 viruses have similar antigens (18), the HI antibody to A/Hong Kong/1/68 exceeded its initial levels after the yak was infected with A/England/42/72 virus. The A/England/42/72 antibody that appeared after infection with A/Hong Kong/1/68 virus was due to cross-reactions. However, the titre increased in 6 days and reached the level seen with A/Hong Kong/1/68 antibody at 23 days p.i. (Fig. 1). No respiratory symptoms were observed after the A/England/42/72 challenge. The RD test, which measured antibodies against both viruses, was strongly positive after infection with A/Hong Kong/1/68 virus; then, after a decline, it became strongly positive again 32 days after infection with A/England/42/72 virus (Fig. 1). The RD test was negative 144 days after the first virus had been given.

Since A/Prague/1/56 antibodies were found to be present in 3 horses in Nepal (6), the susceptibility of the yak to this equine virus was also tested. Malaise and anorexia, without respiratory symptoms or a raised temperature, were observed on the 5th day, and specific antibody was detected on the 7th day (Fig. 1). Attempts to isolate influenzaviruses from the nasal cavity during the first 6 days failed in all 3 challenge experiments. Neutropenia and attacks of haemolysis associated with *Babesia bigemina* followed each influenzavirus inoculation (24).

Naturally-occurring antibody to the H3N2 antigens was found not only in the crossbred yak, but also in cattle (6 positive/9 tested) and water buffaloes (2/2) in 2 locations in Kathmandu, and goats (5/12) and cattle (10/19) in 2 villages in West Bengal, India, as shown by positive RD tests. However, the specificity of water buffalo sera in this test remains to be studied. Single radial diffusion tests provide a sensitive method for measuring the relative concentrations of antibody (18). Thus, a seroconversion in a 6-month-old calf in India was observed in the period June–July 1972. Dogs, chickens, sheep, pigs, and horses were all seronegative (Table 1). The ages of the seropositive cattle ranged from 2 to 42 months, goats from 9 to 24 months, and water buffaloes from 12 to 24 months.

Since HI antibodies to the A/Prague/1/56 virus were present in 3 horses in Nepal, other species were also tested for these antibodies. Chickens (0/41), goats (0/77), cattle (0/20), dogs (0/6), and humans (0/540) were all negative. Experimentally, it has been shown that A/equine 2/Miami/63 (Heq2Neq2) antigen would cross-react with 50% of antibody stimulated by A/Hong Kong/1/68 virus (20). As was expected, low levels of antibodies were detected to the A/equine 2/Miami/63 antigen in 12% (37/318) of the human sera tested. In all probability, these results indicate cross-reactions of A/equine 2/Miami/63 antigen with A/Hong Kong/1/68 antibody rather than primary infections of people with this equine virus. No HI antibody was found in human sera (0/134) when tested with the following avian antigens: A/turkey/Massachusetts/65 (Hav6N2), A/quail/Italy/1117/65 (Hav2Neq2), and A/duck/England/56 (Hav3Nav1). Sera from 47 horses and mules were tested with these 3 avian antigens and with A/chicken/Scotland/59 (Hav5N1); no antibodies were found.

DISCUSSION

Although A/Hong Kong/1/68 and A/England/42/72 influenzaviruses are closely related (18), these challenge experiments in the yak show that, in contrast to what happens in man (7), the former virus did not protect against infection with the latter virus. However, it was shown that the yak produced antibody against A/Hong Kong/1/68 as well as A/England/42/72 when infected with A/England/42/72 virus. Thus, like man (5) and ferrets (21), the yak had an antibody response that illustrated " the doctrine of original antigenic sin".

The presence of naturally acquired H3N2 antibody in the yak–zebu crossbred and the susceptibility of the yak to both of the H3N2 viruses, as well as the equine virus A/Prague/1/56, suggest that yaks should also be considered as possible hosts for recombinant strains of influenza. Yaks occupy the same huts as people and perhaps live closer to man than any other animal in the high altitude areas of Nepal and Tibet.

There is also a close association of people, cattle, water buffaloes, and goats in the lower altitude regions of Nepal and India. This relationship was assessed by comparing the viruses isolated from 6 species of animals (dogs, chickens, cattle, goats, monkeys, and house-crows) in 1972 with those isolated from infants (1, 2) in 1967–68 in the same village (Singur, West Bengal). The distribution of the viral groups (echoviruses, Coxsackie B, adenoviruses, polioviruses, and reoviruses) was the same for the animals and infants (25), these viruses being present in 33 of 226 animals (14%). The antibody studies reported here also showed that cattle and goats in Singur were frequently infected with human influenzavirus (Table 1). Thus, the close contacts between people and animals in Nepal and India may provide suitable environmental conditions to allow genetic recombination of animal and human influenzaviruses to take place. Poliovirus 1 was isolated from the nasal cavities of a goat and a calf in Ichag, West Bengal, but myxoviruses were not recovered from any of 3 species in 193 attempts (Graves & Oppenheimer, unpublished observations).

Other animals such as dogs (13, 15), cats, monkeys (14, 15), pigs (10, 16), horses (8), chickens (23), cattle (4), and water-birds (22) are susceptible to A/Hong Kong/1/68 influenzavirus. HI antibodies to A/England/42/72 influenzavirus were detected in free-living monkeys on Carey Island, Malaysia, in June 1969 (6); this was 25 months prior to the first isolation of this virus from humans (18) and 3 years before serological evidence indicated that the virus was in Nepal (19). HI antibodies to avian and equine influenza strains were not found in human or animal sera, except in 3 horses, but 4 species of animals had antibodies to human strains of influenzavirus (Table 1). Perhaps animals rather than man are the more likely hosts for genetic recombination between influenzaviruses. Work on identifying which of the many possible species including man (3) may be most frequently involved in producing pandemic strains has just begun.

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RÉSUMÉ

RÉCEPTIVITÉ D'UN YACK À DES VIRUS GRIPPAUX A ET PRÉSENCE D'ANTICORPS H3N2 CHEZ DES ANIMAUX AU NÉPAL ET EN INDE

Des anticorps, acquis naturellement, actifs contre les antigènes grippaux humains H3N2 ont été découverts chez un hybride de yack et de zébu au Népal. Des inoculations successives de virus A/Hong Kong/1/68 (H3N2), A/England/42/72 (H3N2) et A/Prague/1/56 (Heq1Neq1), pratiquées chez un yack dépourvu d'anticorps antigrippaux, ont suscité une réponse immunitaire à l'égard des virus inoculés. Des épreuves de diffusion radiale ont montré que des bovins et des chèvres au Bengale Occidental (Inde) ainsi que des buffles et des bovins à Katmandou (Népal) étaient porteurs d'anticorps dirigés contre les antigènes H3N2. Les épreuves d'inhibition de l'hémagglutination n'ont pas permis de déceler des anticorps actifs contre les virus grippaux équins dans des sérums d'hommes, de chèvres, de bovins, de poulets ou de chiens ni d'anticorps actifs contre les virus de la grippe aviaire dans des sérums humains ou équins.

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