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Antibody Response in Certain Domestic Animals to Influenza Viruses. (29288)

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Myxovirus immune sera are commonly produced in such laboratory animal hosts as chicken, ferret, guinea pig and rabbit. Increased epidemiological and diagnostic studies necessitate larger supplies of quality reference and diagnostic sera. An answer to this problem may be utilization of suitable larger animals where volume production with economy of manipulation and material can be achieved. Anti-influenza sera to human strains of virus have been produced in the horse(1). Recently, production of typing antisera in lambs inoculated by the intratracheal route with 3 type A influenza viruses has been reported(2). The present report summarizes a study undertaken to follow antibody response in goat, sheep and burro sera after immunization with influenza viruses.

Materials and methods. Viruses. The following influenza strains were employed as immunizing antigens: A2/Japan/305/56 ($E_4F_1M_3E_{23}$), B/Great Lakes/1739/54 ($E_4M_{10}E_{43}$), A/PR/8/34 ($F_8M_{593}E_{169}$), A1/FM/1/47 ($M_{37}E_{14}M_3E_{50}$), A/Swine/1976/31 ($M_{38}E_{45}$), B/Lee/40 ($F_8M_{137}E_{178}$) and C/Taylor/1233/47 (XE_{15}).* All strains were used for immunization of goats. The A2/Japan/305/57 and B/Great Lakes/1739/54 strains were used for sheep while only the A2/Japan/305/57 strain was used for immunization of the burro.

Immunizing antigens. These consisted of infected allantoic fluids treated by 2 cycles of adsorption and elution from chicken erythrocytes(3). Influenza type C antigen was untreated infected amniotic fluid. The materials were adjusted by dilution or by concentration (4) to contain approximately 200 hemagglutinating units.

Immunization and bleeding. Goats (Alpine cross, Nubian, Saanen and Toggenburg types) weighed between 55 and 125 lb, sheep (Suffolk and Rambouillet-Hampshire types) weighed 49 and 96 lb and a Mexican burro weighed 285 lb. All animals were inoculated intravenously at 0 time and again on day 7 and 35. The goat immunized with B/Lee/40 virus was also inoculated on days 56 and 77. Each inoculation consisted of 20 ml of virus material for goats and sheep and 40 ml of virus material for the burro. Bleedings were taken prior to immunization and at least 10 samples were collected from each animal at

* The letters E, F and M and the numerals designated in subscripts indicate passage history in the egg, ferret and mouse respectively. The complete history for C/Taylor/1233/47 virus is unknown except for a record of 15 egg passages.

TABLE I. Antibody Response in Goats, Sheep and Burro to Influenza Immunizing Antigens.

Immunizing virus	Homologous maximum HI titers				Homologous maximum CF titers			
		Inoculation				Inoculation		
	Pre-	1st	2nd	3rd	Pre-	1st	2nd	3rd
A. Goats								
A2/Japan/305/57	<10	<10* (7)	320 (7–14)	1280 (7, 10)	<4	<4 (7)	32 (7, 10)	128 (7, 10)
B/Great Lakes/1739/54	ND	40 (7)	640 (7)	640 (7)	ND	<4 (7)	64 (7)	32 (7, 10)
A/PR/8/34	<10	40 (7)	1280 (7–14)	5120 (7, 10)	<4	<4 (7)	16 (7, 10)	32 (7, 10)
A/FM/1/47	<10	40 (7)	2560 (10)	2560 (7, 10)	<4	<4 (7)	4 (7–21)	32 (7, 10)
A/Swine/1976/31	<10	<10 (7)	1280 (7)	2560 (7, 10)	<4	<4 (7)	32 (7)	32 (7, 10)
B/Lee/40†	<10	<10 (7)	20 (14, 21)	160 (7, 10)	<4	<4 (7)	<4 (7–28)	8 (7, 10)
C/Taylor/1233/47	<10	2560 (7)	5120 (7, 10)	5120 (10)	<4	64 (7)	NS	NS
B. Sheep								
A2/Japan/305/57	<10	<10 (7)	640 (7)	2560 (7)	<4	<4 (7)	64 (7)	256 (7, 10)
B/Great Lakes/1739/54	<10	20 (7)	640 (7, 10)	2560 (7)	<4	<4 (7)	16 (7–28)	NS
C. Burro								
A2/Japan/305/57	<10	160 (7)	320 (7–14)	1280 (14)	AC	AC	AC	AC

ND = test not done; NS = nonspecific reactivity; AC = anticomplementary.

* First figures represent reciprocals of initial serum dilutions; figures in parentheses represent day(s) maximum response noted following inoculation of virus.

† A titer of 1:640 by HI obtained seven days following fifth inoculation of immunizing antigen (day 84).

periodic intervals thereafter.

Serologic procedures. Conventional methods for hemagglutination-inhibition (HI) test(5) and complement fixation (CF) test (6) were followed. Sera were treated with potassium periodate(7) for use in the HI test when A2/Japan/305/57 virus served as antigen except sera from those animals whose preimmunization sera contained no nonspecific inhibitor. All bleedings collected from each immunized animal were tested simultaneously with the homologous antigen.

Results. Normal sera. Normal sera from 13 goats, 10 sheep and 10 burros were heated at 56°C for 30 minutes and tested for evidence of nonspecific inhibition by HI test. Inhibition at 1:20 dilution was encountered in 10 of 13 goat sera and in dilutions up to 1:40,960 in all burro sera when A2/Japan/305/57 virus was used as antigen. This condition was eliminated with periodate but not with trypsin treatment. Inhibition of this virus was not encountered with normal sheep sera. Wang and Lin(8) also encountered low levels of nonspecific inhibitor in normal goats but not in normal sheep sera. B/Great Lakes/1739/54 virus was not inhibited by any of the 33 normal sera studied.

Immunized animals. With the exception of B/Lee/40 virus, it may be seen in Table I that 2 or 3 intravenous injections of any of the viruses studied resulted in HI titers of 1:640 or greater in the goat, sheep or burro. In the case of the B/Lee/40 virus immunized goat, it was necessary to administer 5 doses of immunizing antigen to attain this titer on day 84. CF titers were much lower in the immunized goats and sheep; burro serum was found to be anticomplementary. From Table II it may be seen that heterologous antibody

TABLE II. Heterologous Antibody Response at Maximum Homologous HI Titers.

Antisera		Influenza virus antigens*							
		A2/Japan/305/57	B/GL/1739/54	A/PR/8/34	A/FM/1/47	A/Swine/1976/31	B/Lee/40	B/Md/1/59	C/Taylor/1233/47
A2/Japan/305/57	Goat	*1280*	20	40	40	20	<10	40	<10
	Sheep	*2560*	<10	20	20	<10	<10	<10	<10
	Burro	*1280*	<10	<10	<10	<10	<10	<10	<10
B/GL/1739/54	Goat	<10	*640*	ND	ND	ND	20	80–320	<10
	Sheep	<10	*2560*	<10	<10	<10	80	1280	<10
A/PR/8/34: Goat		<10	40	*5120*	80	<10	<10	10	<10
A/FM/1/47: Goat		<10	<10	40	*2560*	20	<10	20	<10
A/Swine/1976/31: Goat		<10	<10	20	80–160	*2560*	<10	<10	<10
B/Lee/40: Goat		<10	40	ND	ND	ND	*640*	40	<10
C/Taylor/1233/47: Goat		<10	<10	<10	<10	<10	<10	20	*5120*

* Figures represent reciprocals of initial serum dilutions to virus strains indicated. Italicized figures denote peak homologous titers realized in the immunization series.

ND = test not done; however, other sera selected close in the immunization series to those giving peak homologous titers were <10 with these three antigens.

responses when encountered were generally of a low order, except in the B/Great Lakes/1739/54 goat and sheep, where high titers were found with B/Maryland/1/59 antigen. There is a general tendency toward reduced specificity when multiple doses of influenza virus immunizing antigen are used for production of antisera. In animals receiving 3 doses of B/Great Lakes/1739/54 immunizing antigen, almost identical titers were measurable with the distinct, but related, B/Maryland/1/59 virus. However, following the second dose of antigen, an 8-fold difference in titers measurable with the two viruses was noted. From a practical viewpoint, the optimal bleeding time for minimal heterologous responses can be determined by periodic serologic testing during the course of immunization.

Summary. The antibody response measured by HI and CF tests in 7 goats, 2 sheep and a Mexican burro was followed after inoculation with influenza type A, B and C viruses. Volume production of useful hemagglutination-inhibiting antisera in these animals is possible. Nonspecific inhibitor to Asian influenza virus in normal goat and burro sera was eliminated by periodate treatment.

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