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SUSCEPTIBILITY OF MINK TO CERTAIN VIRAL ANIMAL DISEASES FOREIGN TO THE UNITED STATES

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Abstract: Mink *(Mustela vison)* were inoculated with viruses: African horse sickness (AHS), African swine fever (ASF), bovine herpes virus II (BHV2), foot-and-mouth disease (FMD), goat pox (GP), hog cholera (HC), peste des petits ruminants (PPR), rinderpest (RP), swine vesicular disease (SVD), vesicular exanthema of swine (VES) and vesicular stomatitis (VS). Their susceptibility was measured by development of clinical signs, virus isolation and detection of precipitin and/or virus neutralizing antibodies. SVD virus produced a lesion in one mink. Virus was isolated from mink inoculated with SVD, FMD and BHV2. Neutralizing and/or precipitin antibodies were detected in mink inoculated with ASF, FMD, GP, RP, SVD and VS viruses. Mink were not susceptible to AHS, HC, PPR and VES viruses.

INTRODUCTION

Experimental infection of certain wild animals with some viruses of domestic livestock foreign to the United States are known to produce acute infection with lesions. [3,5,6,14,15] White-tailed deer *(Odocoileus virginianus)* [5,6] and white-collared peccaries *(Tyassu tajucu)* [3] are susceptible to rinderpest (RP) virus. White-tailed deer were also susceptible to foot-and-mouth (FMD) disease virus. [9] On the other hand, one-toed pig *(Sus Scrofa)* were extremely susceptible to viruses of vesicular exanthema of swine (VES), vesicular stomatitis (VS), African swine fever (ASF), swine vesicular disease (SVD), hog cholera (HC) and FMD but not to RP virus. [15] White-collared peccaries developed mild clinical signs with FMD, VS, VES and HC viruses but were resistant to African swine fever (ASF) virus. [3] Armadillos *(Dasypus novemcinctus)* were susceptible to FMD but not to ASF, HC, RP, VES and VS viruses. [14] Indian squirrels *(Funambulus pennanti)* were recently shown to be extremely susceptible to FMD by intramuscular or intradermal route but failed to cause infection by oral route or by contact. [13]

There are large populations of mink in the wild and in captivity in the United States. The role of mink as a carrier of the viral diseases foreign to the United States is not known. The purpose of this experiment was to establish the susceptibility of mink to AHS, ASF, BHV2 also known as bovine herpes mammilitis or allerton, FMD, goat pox (GP), HC, peste des petits ruminants (PPR), RP, SVD, VES and VS viruses (Table 1).

MATERIALS AND METHODS

Test Animals

The test group consisted of 15 mink. The mink were divided into four groups. Three groups, each consisting of four mink, were inoculated with viruses. The mink were anesthesized by intramuscular inoculation with a mixture of xylazine 0.3 mg [2] and ketamine

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TABLE 1. Experimental design for testing susceptibility of mink to certain animal diseases foreign to the United States.

Group No.	Virus[a]	Inoculation Day	Virus Type	Virus Doses[b]	Site of Inoculation	Type of Serological Test Used[c]	Biological System Used for Virus Isolation and VN Test[d]	References
1	FMD	0	O_1	$1\times10^{7\ 9}$ MLD_{50}	Foot Pad & Tongue	VN and CF	Mice 3-5 day old	3, 9
	RP	14	Kabete "O"	5×10^{3} $TCID_{50}$	Muscular	VN	Vero cell line	5
	HC	28	Ames	1×10^{4} PLD_{50}	Muscular	FA plaque inhibition	PK^{15} cell line	Text
	SVD	42	UK	5×10^{5} $TCID_{50}$	Foot Pad	VN and CF	MVPK cell line	14
2	AHS	0	9	$5\times10^{7\ 1}$ $TCID_{50}$	Muscular	VN and CF	Vero cell line	14, 15
	GP	14	Held, Turkey	$1\times10^{4\ 5}$ $TCID_{50}$	Muscular	VN and AGD	Primary lamb testis	1
	VS	28	NJ	$\times10^{6\ 5}$ EID_{50}	Foot Pad	VN	7 day old fertile chicken egg	14
3	ASF	0	Tengani	1×10^{5} PLD_{50}	Muscular	Immunoelectroosmophoresis (IEOP)	Swine buffy coat	7, 14, 15
	BHV2	14	Allerton	$2\times10^{6\ 6}$ $TCID_{50}$	Intravenous	VN	Primary bovine kidney	
	VES	28	A_{48}	$5\times10^{7\ 8}$ $TCID_{50}$	Foot Pad	VN	Vero cell line	14
	PPR	42		$5\times10^{3\ 3}$ $TCID_{50}$	Muscular	VN	Vero cell line	6
4	None	—	—	—	—	—	—	—
5	FMD		A_5	$1\times10^{7\ 5}$ MDL_{50}	Foot Pad & Tongue	VN and CF	Mice 3-5 day old	3, 9, 14

[a]FMD = foot-and-mouth disease; RP = rinderpest; HC = hog cholera; SVD = swine vesicular disease
AHS = African horse sickness; GP = goat pox; VS = vesicular stomatitis; ASF = african swine fever;
BHV2 = bovine herpes virus II; VES = vesicular exanthema of swine; PPR = peste des petits ruminants

[b]MLD = mouse lethal dose; TCID = tissue culture infective dose; PLD = pig lethal dose; EID = egg infective dose

[c]VN = virus neutralization; FA = fluorescent antibody; CF = complement fixation; AGD = agar gel diffusion

[d]Vero = green mondey kidney; PK^{15} and MVPK = pig kidney

hydrochloride 1.8 mg. [3] Three uninoculated mink of the 4th group served as controls. All mink of groups 1, 2, 3 and 4 were inoculated with FMD type A_5 virus and called group 5. All inoculated mink were observed for 14 days postinoculation (DPI) for clinical signs of infection. Blood samples for virus isolations were taken at 2, 3 and 7 DPI. The blood samples were diluted 1:10 and 1:100 in Hank's BSS before inoculating into tissue culture and animals, respectively. Sera for serological tests were taken at 14 DPI from the jugular vein.

Cell Cultures and Media

Vero (green monkey kidney) cell line, pig kidney cell lines (PK 15 and MVPK), primary lamb testis, primary bovine kidney and swine buffy coat were used.

MEM (Eagle) with Hank's salt, L-glutamine, 2.2g $NaHCO_3$/L, penicillin, (100 IU/ml), streptomycin (50 μg/ml) and kanamycin (10 μg/ml) were used throughout the experiment. Ten percent bovine fetal calf serum was added to medium used for growing tissue culture and 5% serum for maintenance of the cultures.

Viruses Inoculation, Isolation And Serology

Table 1 describes the experimental design.

Fluorescent Antibody Plaque Inhibition (FAPI) Test

FAPI test was used for hog cholera (HC). Sera taken at 14 DPI were diluted from 1:4 to 1:64 in phosphate buffered saline (PBS) pH 7.4. A 0.2 ml of each dilution of serum was mixed with 0.2 ml of viruses in PBS pH 7.4 containing 100 plaque forming unit (PFU) of HC virus. The virus-serum mixture was incubated in a 37 C water bath for 30 min. PK 15 cell line on cover slips (50 mm $\times$ 10.6 mm) in Leighton tubes were inoculated with 0.2 ml of virus-serum mixture. The virus was adsorbed for 1 h and washed 3 times with 2 ml of PBS, after which 2 ml of MEM (Eagle) with 5% fetal calf serum was added to each tube. At 18 h after infection, all coverslips were fixed in cold acetone for 10 min. Fluorescein isothiocyanate (FITC) conjugated anti-hog cholera serum was used as described.[8] A dilution of serum that inhibited 90% of the fluorescent plaques was considered the titer of the serum.

RESULTS

Virus Isolation and Clinical Signs

Three viruses were isolated from mink out of the 12 viruses used in the experiment. BHV II (Allerton), FMD type A_5 and SVD viruses were isolated from blood samples (Table 2). SVD and BHV2

TABLE 2. Virus isolation from blood of mink inoculated with animal disease viruses foreign to the United States.

Virus isolated	No. isolated/ No. inoculated	Days postinoculation
BHV2	2/4	2,3
FMD (A_5)	1/15	1
SVD	1/4[a]	2,3

[a]Mink showed a vesicle on right side of upper lip and on pad of left hind foot at 2 DPI

[3] Ketaject, Bristol, Lab., Syracuse, New York 13201, USA.

viruses were isolated at 2 and 3 DPI from one and two mink respectively. FMD type A_5 was isolated from 1 of 15 mink at 1 DPI only.

Other than one mink inoculated with SVD virus, no clinical signs were observed in mink inoculated with all other viruses used in the experiment. Small vesicles appeared on right side of the upper lip and left hind foot of that mink at 2 DPI. They were healed by 5 DPI. Virus was isolated from blood of this mink. No attempt was made to isolate virus from the vesicles.

Serologic Reactions

At 14 DPI in mink sera, antibodies were detected to ASF, FMD types 0_1, and A_5, GP, RP, SDV and VS viruses (Table 3). Antibody was detected in 1 out of 4 mink to FMD type O, and ASF viruses. High virus neutralizing antibody titers were observed to RP (1:16 to > 1:64), SDV (1:16 to 1:64), VS (>1:64) and FMD type A_5 (1:4 to >1:64) viruses. Precipitin antibody to GP virus was observed in all four mink and 4 of 15 mink had a complement fixing titer of 1:16 to FMD type A_5 virus. Serum antibody was not

TABLE 3. Serologic response of mink to infection with various exotic animal disease viruses.

		Serological Tests	
		No. with antibody	No. inoculated
Group	Viruses[a]	A*	B**
1	FMD (O_1)	1/4	1/4
	RP	ND	3/4
	HC	ND	0/4
	SVD	2/4	4/4
2	AHS	0/4	0/4
	GP	4/4	1/4
	VS	ND	4/4
3	ASF	1/4	ND
	BHV2	0/4	0/4
	VES	0/4	0/4
	PPR	ND	0/4
4	None	0/3	0/3
5	FMD (A_5)	4/15	9/15

[a]FMD = foot-and-mouth disease; RP = rinderpest; HC = hog cholera;
SVD = swine vesicular disease; AHS = african horse sickness; GP = goat pox;
VS = vesicular stomatitis; ASF = african swine fever;
BHV2 = bovine herpes virus II; VES = vesicular exanthema of swine;
PPR = peste des petits ruminants

A = IEOP or AGD or CF tests;

B = virus neutralization tests;

ND = not done

* = Complement fixation (CF) titer for preinfected sera was <1:8, the titer of positive sera for homologous virus varied from 1:8 to 1:32.

** = Virus neutralization titer of preinfected sera was <1:4, the titer of positive sera for homologous virus varied from 1:4 to >1:64.

observed to HC, AHS, BHV2, VES and PPR viruses.

DISCUSSION

The most frequent method of transmission of FMD in enzootic areas is by direct contact among susceptible animals.[4] Mink were found to be partially susceptible to FMD. FMD type A_5 virus was isolated in 1 of 15 mink but type O virus was not isolated. Serologic studies also indicated a partial susceptibility because antibody was induced in 9 of 15 mink inoculated with type A_5 and 1 of 4 mink with type O_1. These limited studies do indicate that mink could carry and become infected with FMD virus, especially type A_5, and could contaminate feed by their excrement. However, the ability to transfer FMD to susceptible domestic animals would be minimal. Mink are shy and do not routinely mix with domesticated animals.

BHV2 virus produced a detectable viremia in 2 of 4 mink at 2 and 3 DPI. Therefore, mink must be considered susceptible and capable of transmitting BHV2 virus even though no lesions or morbidity were detected. The possibility that a carrier state could develop in mink with BHV2 virus must also be considered because detectable antibodies fail to develop within 14 days.

SVD is a newly isolated virus that has been responsible for extensive lesions in swine in Europe and Asia.[2,10,11] The virus has a limited host spectrum. Cattle, rabbit, chicken, hamster, sheep and guinea pig are all resistant to SVD virus.[2,10] However, mink are susceptible. All mink had high antibody level to SVD virus and one of four inoculated mink that was viremic at 2 and 3 DPI had a vesicule on the right side of the upper lip. Based on clinical signs, mink must be considered in the epizootiology of SVD outbreaks on farms.

Mink inoculated with RP, GP and VES viruses developed antibody; however, virus was not isolated. The susceptibility of mink to these viruses cannot be determined, because antibody could be either a response to the virus inoculum or to virus replication. Mink are resistant to HC, AHS, VES and PPR viruses because neither virus nor antibody was detected.

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