Effect of strain and serotype of vesicular stomatitis virus on viral shedding, vesicular lesion development, and contact transmission in pigs

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Objective—To determine whether pigs can be infected with strains of vesicular stomatitis virus New Jersey (VSV-NJ) and vesicular stomatitis virus Indiana (VSV-I) isolated during recent vesicular stomatitis outbreaks that primarily involved horses in the western United States and determine the potential for these viruses to be transmitted by contact.

Animals—128 pigs.

Procedure—Pigs were challenged with VSV-NJ or VSV-I from the 1995 and 1997 outbreaks of vesicular stomatitis in the western United States, respectively, or with VSV-NJ (OS) associated with vesicular stomatitis in feral pigs on Ossabaw Island, Ga. Pigs (3/group) were inoculated with each virus via 3 routes and evaluated for viral shedding, seroconversion, and the development of vesicular lesions. In another experiment, the potential for contact transmission of each virus from experimentally infected to naïve pigs was evaluated.

Results—Infection of pigs was achieved for all 3 viruses as determined by virus isolation and detection of seroconversion. In inoculated pigs, all 3 viruses were isolated from multiple swab samples at concentrations sufficient to infect other pigs. However, compared with results obtained with the 2 VSV-NJ strains, viral titers associated with VSV-I were low and the duration of virus shedding was reduced. Results from the contact transmission trials were consistent with these results; virus transmission was detected most frequently with the VSV-NJ strains.

Conclusions and Clinical Relevance—Pigs can be infected with VSV-NJ and VSV-I. Differences in the extent of viral shedding and potential for contact transmission were apparent between serotypes but not between the VSV-NJ strains investigated. (*Am J Vet Res* 2004;65:1233–1239)

Vesicular stomatitis, which is caused by related Vesiculoviruses in the family Rhabdoviridae, is a disease of cattle, horses, and swine. In the United States, **vesicular stomatitis virus New Jersey (VSV-NJ)** and **vesicular stomatitis virus Indiana (VSV-I)** have been associated with vesicular stomatitis as recently as 1997 and 1998, respectively.[1] Vesicular stomatitis is an important livestock disease that can cause both direct and indirect economic losses to livestock producers.[2] Indirect economic losses are related to regulatory responses associated with the designation of vesicular stomatitis as a list A disease by the Office International Des Epizooties (OIE) and its clinical similarity with other list A diseases such as foot-and-mouth disease.

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Although economically important, the epidemiology of vesicular stomatitis is not well defined. Transmission of VSV-NJ can occur via biological[3-8] and mechanical vectors[9] and as a result of animal-to-animal contact,[10-12] but the relative importance of these routes in the spread of this virus among livestock populations is unclear. Less is known about potential transmission mechanisms for VSV-I. Biological vectors of VSV-I have been described,[13,14] but results of epidemiologic studies[15,16] to date suggest that transmission mechanisms for this virus may differ from those associated with VSV-NJ.

Domestic pigs provide a reliable experimental system for investigation of the potential for both contact and mechanical vector transmission of VSV-NJ in domestic animal populations.[17-20] Attempts at experimental infection of domestic pigs with VSV-NJ originally isolated from Ossabaw Island, Ga, (designated VSV-NJ [OS]) have demonstrated that swine can be infected by various routes that are compatible with mechanical and contact transmission[17-19] with as little as $10^{0.7}$ **median tissue culture infective doses ($TCID_{50}$)** of virus.[17] The virus can be detected in swabs of the nasal planum, nasal cavity, and tonsil of the soft palate and samples of saliva and feces but is not detected in blood.[17-20] Viral concentrations from swabs can exceed $10^{5.1}$ $TCID_{50}$, and VSV-NJ (OS) can be detected from individual animals for as long as 7 consecutive days.[19] The development of vesicular lesions is dependent on both route of inoculation and viral dose.[18-20] Contact transmission can occur within 24 hours of exposure to an infected animal but has been determined only when vesicular lesions were present.[20] All of these findings suggest that contact and mechanical transmission may represent important transmission routes once this virus is introduced into a domestic animal population.

Presently, there is little information regarding VSV-I infection in pigs. Results of an experimental study[10] have indicated that pigs are susceptible and will develop vesicular lesions at the site of inoculation.

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Secondary lesions (ie, vesicular lesions detected in areas other than the original lesion site) rarely develop. There have been no reported cases of naturally occurring VSV-I infection in pigs in the United States, but antibodies against VSV-I have been detected in pigs in Central America.[21] No information is available on the extent of viral shedding associated with VSV-I infection in pigs.

Recent outbreaks of vesicular stomatitis that largely affected horses in the western United States involved both VSV-NJ and VSV-I. The VSV-NJ isolated during these recent outbreaks is genetically distinct from VSV-NJ (OS).[22] The purpose of the study reported here was to determine whether pigs can be infected with strains of VSV-NJ and VSV-I isolated during recent vesicular stomatitis outbreaks that primarily involved horses in the western United States and determine the potential for these viruses to be transmitted by contact.

Materials and Methods

Swine—One hundred twenty-eight commercially available mixed-breed pigs (weight, approx 10 kg) were included in the study. All pigs were seronegative against VSV-NJ and VSV-I, as indicated by results of serum neutralization tests, and were housed in insect-proof isolation units in groups of 3 or 4.

Experiment 1 study design—Three previously described inoculation routes, which experimentally result in a full range of clinical (associated with development of vesicular lesions) and subclinical (not associated with development of vesicular lesions) infections with VSV-NJ in pigs, were used in this portion of the study.[18,19] These included ID inoculation of the apex of the snout, application of virus to a scarified area of the oral mucosa, and application of virus to intact oral mucosa. In pigs, infection via ID inoculation of the snout and application of virus to scarified oral mucosa results in the development of vesicular lesions, viral shedding via the tonsils and nasal cavity, and seroconversion.[18,19] Infection by application of virus to intact oral mucosa results in viral shedding and seroconversion but is not associated with development of vesicular lesions.[18,19] For each route, 3 viruses were used: VSV-NJ (OS), an isolate of VSV-NJ[a] obtained during 1995 from horses in Colorado (designated VSV-NJ [CO]), and an isolate of VSV-I[b] obtained during 1997 from horses in New Mexico (designated VSV-I [NM]). The inoculum for the 2 VSV-NJ strains consisted of 100 µL of vesicular fluid collected from a pig that had been inoculated ID in the snout. The VSV-I inoculum for this initial experiment consisted of infected tissue culture fluid derived from infected Vero cell culture. Three viral doses were used: approximately 10^6, 10^4, and 10^2 $TCID_{50}$/100 µL. Challenges with the 2 lower doses were administered only via ID inoculation of the snout. Three pigs were infected for each combination of route, virus, and dose (ID inoculation of the snout only). An additional group of 3 pigs was inoculated with 100 µL of **minimum essential medium (MEM)** as a negative control.

All pigs were examined daily on **postinoculation days (PIDs)** 1 through 14 for clinical signs of disease. Samples for virus isolation were obtained from pigs on PIDs 1, 3, 5, 7, 9, and 12. Swab specimens of the nasal planum, nasal cavity, tonsil of the soft palate, saliva, and feces were collected in 1 mL of transport medium consisting of MEM supplemented with antimicrobials (1,000 units of penicillin G, 1 mg of streptomycin, 0.25 mg of gentamicin sulfate, 0.5 mg of kanamycin monosulfate, and 2.5 µg of amphotericin B/mL).[c] Sera for virus isolation were collected from blood samples on PIDs 1, 3, and 5. Sera for serologic testing were obtained from blood samples collected from all swine prior to inoculation and just prior to euthanasia. Pigs were euthanatized on PID 14 via IV administration of an overdose of sodium pentobarbital.

Experiment 2 study design—Two inoculation routes, including ID inoculation of the snout and application of virus to scarified oral mucosa, were used in this portion of the study. Viral inocula (10^6 $TCID_{50}$/100 µL) were identical to those used in experiment 1, except the inoculum of VSV-I (NM) consisted of infective vesicular fluid rather than infective tissue culture medium. Each contact trial involved commingling 1 inoculated pig with 3 contact pigs, as described.[20] Contact pigs were introduced into the pen in which the inoculated pig was housed 24 hours after the experimental inoculation. For VSV-NJ (CO) and VSV-I (NM), 4 contact trials were completed for each route of inoculation. Because consistent contact transmission had been demonstrated with VSV-NJ (OS) in swine,[20] only 2 contact trials were conducted for each route with that virus. Pigs were observed daily for clinical signs of disease; on **postcontact days (PCDs)** 1, 3, 5, 7, 9, and 12, swab specimens of the nasal cavity and tonsil of the soft palate were collected in 1 mL of the same transport medium used in experiment 1. Sera for serologic testing were obtained from blood samples collected from all swine prior to inoculation or contact with inoculated animals and just prior to euthanasia on PCD 12. Pigs were euthanatized on the final day of the experiment via IV administration of an overdose of sodium pentobarbital.

Virus isolation and serologic testing—Virus isolation from swabs was attempted by use of Vero cells on 12-well culture plates. Briefly, swabs in transport medium were vortexed and centrifuged at 1,500 X g for 15 minutes. Individual wells containing confluent monolayers of Vero cells in 2 mL of maintenance medium (MEM supplemented with 3% fetal bovine serum and antimicrobials at a concentration of 100 units of penicillin, 0.1 mg of streptomycin, and 0.25 µg of amphotericin B/mL) were inoculated with 100 µL of supernatant from swab specimens or 100 µL of serum. All virus isolations were completed immediately after sample collection, and residual supernatant from the samples was frozen at –70°C. If a cytopathic effect in cell culture was not observed within 72 hours, results for the sample were considered negative (no virus detected). If a cytopathic effect was observed (positive result), cell culture fluids were collected and evaluated immunohistochemically to confirm the presence of VSV-NJ and VSV-I.[17] For all samples with positive results, virus concentrations were quantified by endpoint titration in Vero cells with the stored frozen supernatant.[17] Sera were assessed for antibodies against VSV-NJ and VSV-I via serum neutralization, as described.[17] A 4-fold or greater increase in antibody titer (values reported as the reciprocal of highest dilution neutralizing 1,000 $TCID_{50}$ of virus), compared with baseline values, was considered indicative of seroconversion.

Results

Experiment 1—Virus was not isolated from any serum samples obtained from pigs after inoculation with any of the 3 viruses via any route. Also, virus was not detected in fecal samples obtained from pigs after inoculation with any of the 3 viruses via any route, except in a sample from 1 pig that received VSV-NJ (CO) at a dose of $10^{5.9}$ $TCID_{50}$ via application to a scarified area of oral mucosa (viral titer of < 2.3 $TCID_{50}$). After inoculation with VSV-NJ (OS), infected pigs were identified by positive virus isolation results (from swabs of the nasal planum or cavity, saliva, or tonsil) or detection of seroconversion in all route-dose treatment

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groups (**Table 1**). Viral shedding was detected as early as PID 2 in pigs after ID inoculation in the snout (inoculum, $10^{5.9}$ $TCID_{50}$) and PID 1 in pigs that received application of virus to a scarified area of the oral mucosa. Viral shedding was detected for as many as 4 consecutive days, and seroconversion was detected in all pigs from which virus was isolated.

After inoculation with VSV-NJ (CO), infected pigs also were identified by positive virus isolation results or detection of seroconversion in all route-dose treatment groups. After ID inoculation in the snout, viral shedding of VSV-NJ (CO) was detected as early as PIDs 2 and 3 in pigs receiving inocula of $10^{5.9}$ $TCID_{50}$ and $10^{3.6}$ $TCID_{50}$, respectively; furthermore, shedding was detected for a maximum of 4 and 5 days, respectively. Treatment of the scarified oral mucosa (inoculum, $10^{5.9}$ $TCID_{50}$) resulted in detectable virus shedding on PID 1, which persisted for a maximum of 6 days. Seroconversion was identified in all but 1 pig from which VSV-NJ (CO) was isolated; the exception was a pig that was inoculated via a scarified area of oral mucosa. In that pig, a vesicular lesion developed and virus shedding was detected for 6 consecutive days, but the pig remained seronegative at PID 14.

After inoculation with VSV-I (NM), infected pigs were identified by results of virus isolation or detection of seroconversion in all route-dose treatment groups, except the group receiving ID inoculation of the snout (inoculum, $10^{1.9}$ $TCID_{50}$). After ID inoculation in the snout, viral shedding of VSV-I (NM) was detected as early as PIDs 2 and 4 in pigs receiving inocula of $10^{5.9}$ $TCID_{50}$ and $10^{3.6}$ $TCID_{50}$, respectively; viral shedding was detected for a maximum of 3 days in the former group and only 1 day in the latter. Treatment of the scarified oral mucosa (inoculum, $10^{6.0}$ $TCID_{50}$) resulted in detectable virus shedding on PID 2, which persisted for only 1 day. Seroconversion occurred in all pigs from which virus was isolated, with the exception of 1 pig in the group inoculated via a scarified area of the oral mucosa; virus was isolated from this animal from a single swab specimen (tonsil) on 1 day.

Vesicular lesions developed after inoculation with all 3 viruses (Table 1). After inoculation with VSV-NJ (OS), lesions were detected only in pigs that received an ID inoculum of $10^{5.9}$ $TCID_{50}$ in the snout and those in which virus was applied to a scarified area of the oral mucosa. Vesicles developed at the site of inoculation with VSV-NJ (OS) on PID 2 or 3 in 2 pigs administered an inoculum of $10^{5.9}$ $TCID_{50}$ in the snout; 1 pig developed a vesicle that was 5 mm in diameter, and the other pig developed a vesicle that involved the entire apex of the snout. These vesicles ruptured and appeared to be healing, but on PIDs 7 and 8, new vesicle formation was observed at the edges of the original vesicles in both pigs. By PID 12, the vesiculated areas were healing. All 3 pigs receiving an inoculum of 10^6 $TCID_{50}$ via scarification of the oral mucosa developed multiple vesicles (1 to 5 mm in diameter) at the site of inoculation by PIDs 2 and 3; all affected areas were healing by PID 5.

After inoculation with VSV-NJ (CO), lesions were observed only in pigs receiving ID inoculation in the snout (inocula, $10^{5.9}$ $TCID_{50}$ and $10^{3.6}$ $TCID_{50}$) and those in which the virus was applied to a scarified area of the oral mucosa. On PID 2, both of the pigs receiving $10^{5.9}$ $TCID_{50}$ of VSV-NJ (CO) developed a vesicle at the site of inoculation on the apex of the snout; in 1 pig, the vesicle was 1 cm, and in the other, the vesicle involved the entire apex. By PID 4, both vesicles had ruptured and were healing. One pig inoculated with $10^{3.6}$ $TCID_{50}$ of VSV-NJ (CO) developed a vesicle at the snout inoculation site on PID 3, and this vesicle spread to involve the entire apex of the snout. The vesicle ruptured on PID 6 and was healing on PID 7, but a secondary vesicle developed on the tongue. In another pig in this inoculation group, a vesicle (2 cm in diameter) developed on the edge of the snout in a location other than the inoculation site on PID 8. All 3 pigs inoculated with $10^{5.9}$ $TCID_{50}$ of VSV-NJ (CO) by application of virus to

Table 1—Results of inoculation of pigs* with various doses of vesicular stomatitis virus New Jersey (VSV-NJ) isolated from Ossabaw Island (OS) or Colorado (CO) and vesicular stomatitis virus Indiana (VSV-I) isolated from New Mexico (NM) via ID inoculation of the apex of the snout and application of virus to a scarified or intact area of the oral mucosa.

Inoculation route	Agent	Dose ($TCID_{50}$)	No. of pigs with lesions†	Virus isolations (No. of pigs [maximum titer, $TCID_{50}$/swab])				
				Nasal planum	Nasal cavity	Saliva	Tonsil	Serologic testing (serum neutralization)‡
Snout	VSV-NJ (OS)	$10^{5.9}$	2 (Sn)	3 (4.6)	3 (≥ 5.1)	3 (2.3)	3 (3.6)	3 (≥ 256)
	VSV-NJ (CO)	$10^{5.9}$	2 (Sn)	2 (< 2.3)	2 (2.6)	2 (2.3)	2 (4.4)	2 (≥ 256)
	VSV-I (NM)	$10^{5.9}$	3 (Sn)	3 (< 2.3)	2 (< 2.3)	2 (< 2.3)	2 (< 2.3)	2 (16 – 256)
Snout	VSV-NJ (OS)	$10^{3.3}$	0	0	0	0	0	3 (16 – 256)
	VSV-NJ (CO)	$10^{3.6}$	2 (Sn)	1 (< 2.3)	1 (< 2.3)	2 (< 2.3)	2 (< 2.3)	3 (≥ 256)
	VSV-I (NM)	$10^{3.6}$	2 (Sn [1], O [1])	1 (2.3)	2 (3.6)	1 (< 2.3)	0	3 (64 – 128)
Snout	VSV-NJ (OS)	$10^{1.9}$	0	0	0	0	0	1 (≥ 256)
	VSV-NJ (CO)	$10^{2.0}$	0	0	0	0	0	3 (64 – 256)
	VSV-I (NM)	$10^{1.9}$	0	0	0	0	0	0
Scarified mucosa	VSV-NJ (OS)	$10^{6.0}$	3 (O)	0	0	3 (< 2.3)	2 (3.9)	3 (128 – ≥ 256)
	VSV-NJ (CO)	$10^{5.9}$	3 (O [2], O/Sn [1])	3 (3.1)	3 (3.3)	3 (3.3)	3 (4.3)	2 (≥ 256)
	VSV-I (NM)	$10^{6.0}$	1 (O)	1 (< 2.3)	1 (< 2.3)	0	2 (< 2.3)	1 (≥ 256)
Intact mucosa	VSV-NJ (OS)	$10^{5.9}$	0	0	0	0	1 (< 2.3)	1 (≥ 256)
	VSV-NJ (CO)	$10^{5.4}$	0	0	0	0	0	1 (≥ 256)
	VSV-I (NM)	$10^{5.6}$	0	0	0	0	0	1 (16)
Control	MEM	0	0	0	0	0	0	0

*Each treatment group included 3 pigs, except the group receiving VSV-NJ (CO) via ID inoculation of the snout (inoculum, $10^{5.9}$ $TCID_{50}$) in which 1 pig died of an unrelated cause prior to the experiment. †Lesions developed on the snout (Sn) and in the oral cavity (O). ‡Number of pigs with detectable antibodies (range of neutralization titers [titer equals reciprocal of highest dilution neutralizing 1,000 $TCID_{50}$ of virus]).

$TCID_{50}$ = Median tissue culture infective dose.

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scarified areas of oral mucosa developed vesicles at the site of inoculation by PID 2. These vesicles ruptured by PID 2 or 3, and there was evidence of healing by PIDs 5 through 8. Two of these pigs developed secondary vesicles; 1 pig developed multiple large (> 2 cm) vesicles on the snout on PIDs 3 and 4, and the other developed a small vesicle on the tongue on PID 3.

In all pigs inoculated ID with $10^{5.9}$ $TCID_{50}$ of VSV-I (NM) in the snout, a vesicle (5 mm to 2 cm in diameter) developed at the site of inoculation on PID 2. The smallest of these vesicles was healed by PID 3. In the other 2 pigs, the vesicles ruptured and appeared to be healing via granulation, but on PID 8, new vesiculation developed at the edges of the original vesicle. In 2 pigs inoculated ID with $10^{3.6}$ $TCID_{50}$ of VSV-I (NM), 1- and 1.5-cm vesicles developed at the site of inoculation. These vesicles quickly ruptured and were healing by granulation on PID 6; however, on PID 8, new vesiculation and spreading of vesicles were detected at the edge of the healing vesicles. One pig inoculated with 10^{6} $TCID_{50}$ of VSV-I (NM) via scarification of the oral mucosa developed a small (2 mm in diameter) vesicle at the site of inoculation on PID 3. This vesicle quickly ruptured and was healed by PID 4.

Experiment 2—Transmission of virus from pigs inoculated ID in the snout to contact pigs was detected with all 3 VSV strains (**Table 2**). After exposure to pigs inoculated with VSV-NJ (OS), VSV-NJ (CO), or VSV-I (NM), transmission was confirmed by virus isolation and seroconversion in 3 of 6, 2 of 12, and 1 of 12 contact pigs, respectively. Virus shedding was initially detected in contact pigs on PCD 3 for both VSV-NJ (OS) and VSV-NJ (CO). Transmission of VSV-I (NM) from inoculated pigs to contact pigs was detected from a single contact animal through evidence of seroconversion only. Isolation of VSV-NJ (CO) was obtained once each for 2 pigs that did not seroconvert after contact with VSV-NJ (CO)-inoculated pigs, and VSV-I (NM) was isolated once from 1 pig that did not seroconvert after contact with VSV-I (NM)-inoculated pigs. These isolations could have represented cross contamination and were not regarded as evidence of contact infection.

Transmission of virus from pigs inoculated via application of virus to scarified areas of oral mucosa to contact pigs was detected only for VSV-NJ (OS) and VSV-NJ (CO) (**Table 3**). After exposure to pigs inoculated with VSV-NJ (OS) and VSV-NJ (CO), transmission was confirmed by virus isolation and seroconversion in 3 of 6 and 8 of 12 contact pigs, respectively. Virus shedding was initially detected in contact pigs on PCD 3 for both VSV-NJ (OS) and VSV-NJ (CO). In 1 pig in contact with a VSV-I (NM)-inoculated pig, virus was isolated on 1 occasion but this contact pig did not seroconvert.

All but 3 of the inoculated pigs used in experiment 2 developed lesions at the site of inoculation (Tables 2 and 3). The 3 pigs that did not develop lesions included 2 pigs that were inoculated ID in the snout with VSV-I (NM) and 1 pig that was inoculated with VSV-I (NM) via scarified areas of the oral mucosa. Among the pigs that were inoculated in the snout, vesicles developed on PID 3 in those receiving VSV-NJ (OS), on PIDs 2 through 5 in those receiving VSV-NJ (CO), and on PID 2 in those receiving VSV-I (NM). In pigs that were inoculated via scarified areas of the oral mucosa and subsequently developed lesions, vesicles or erosion and ulceration were observed at the inoculation site on PID 2; only pigs inoculated with VSV-NJ (OS) developed secondary vesicles, and these were observed on PID 5 in 1 pig and on PID 6 in the other.

In contact pigs, lesions developed only after commingling with pigs inoculated ID with VSV-NJ (OS). In 1 of the 2 contact trials performed with this virus, 1 pig developed vesiculation of approximately half of the nasal planum on PCD 7 and another pig had ulceration and moist dermatitis around the preputial orifice, which was first observed on PCD 4. In the second contact trial, ulceration around the preputial opening in 1

Table 2—Result of trials to investigate contact transmission of VSV-NJ (OS), VSV-NJ (CO), and VSV-I (NM) from pigs inoculated ID in the snout with those viruses to virus-naïve contact pigs.

		Inoculated pigs (1/trial)				Contact pigs (3/trial)				
			Virus isolation				Virus isolation		Serologic testing	
Virus	Trial	Location of lesions*	Source†	Duration of shedding (d)	Serologic testing	Lesions (No. of pigs)*	No. of pigs shedding virus	Source†	No. of seropositive pigs	Antibody titer‡
VSV-NJ (OS)	1	Sn	N, S (4.9)	3	≥ 256	Sn, P (2)	2	N, S (≥ 6.1)	2	≥ 256
	2	Sn	N, S (≥ 6.1)	3	≥ 256	P (1)	1	N, S (4.3)	1	≥ 256
VSV-NJ (CO)	1	Sn	S (< 2.3)	1	≥ 256	ND	0	NA	0	NA
	2	Sn	ND	NA	≥ 256	ND	2	N, S (2.6)	0	NA
	3	Sn	ND	NA	≥ 256	ND	1	S (2.9)	1	≥ 256
	4	Sn	S (< 2.3)	1	≥ 256	ND	1	S (4.3)	1	≥ 256
VSV-I (NM)	1	Sn	ND	NA	≥ 256	ND	0	NA	0	NA
	2	ND	ND	NA	128	ND	0	NA	0	NA
	3	Sn	N (< 2.3)	1	≥ 256	ND	1	N (< 2.3)	1	32
	4	ND	ND	NA	≥ 256	ND	0	NA	0	NA

*Vesicular lesions were detected on the snout (Sn) or prepuce (P) or were not detected (ND). †Virus was isolated from swabs of nasal planum or cavity (N) and saliva or tonsil (S); number in parentheses represents the maximum virus titer ($TCID_{50}$/swab). ‡Values of serum neutralization titers (equals reciprocal of highest dilution neutralizing 1,000 $TCID_{50}$ of virus).
NA = Not applicable.

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Table 3—Result of trials to investigate contact transmission of VSV-NJ (OS), VSV-NJ (CO), and VSV-I (NM) from pigs inoculated via application of virus to scarified areas of oral mucosa to virus-naïve contact pigs.

		Inoculated pigs (1/trial)				Contact pigs (3/trial)				
			Virus isolation				Virus isolation		Serologic testing	
Virus	Trial	Location of lesions	*Source†	Duration of shedding (d)	Serologic testing	Lesions (No. of pigs)*	No. of pigs shedding virus	Source†	No. of seropositive pigs	Antibody titer‡
VSV-NJ (OS)	1	0	S (2.3)	3	256	ND	1	S (5.6)	2	32, ≥ 256
	2	0	S (3.6)	3	≥ 256	ND	0	NA	1	64
VSV-NJ (CO)	1	0	N, S (2.9)	5	≥ 256	ND	2	N, S (3.1)	3	≥ 256
	2	0	N, S (5.6)	5	≥ 256	ND	0	NA	0	NA
	3	0	N, S (≥ 6.1)	5	≥ 256	ND	3	N, S (5.9)	3	≥ 256
	4	0	N, S (5.9)	5	≥ 256	ND	1	S (4.3)	2	128, ≥ 256
VSV-I (NM)	1	0	ND	NA	64	ND	0	NA	0	NA
	2	ND	ND	NA	< 8	ND	0	NA	0	NA
	3	0	S (2.3)	3	128	ND	1	S (< 2.3)	0	NA
	4	0	S (< 2.3)	1	≥ 256	ND	0	NA	0	NA

*‡Values of serum neutralization titers for pigs that seroconverted (equals reciprocal of highest dilution neutralizing 1,000 TCID^{50} of virus). *See* Table 2 for remainder of key.

contact pig was first detected on PCD 6. On PCD 12 in the pigs with preputial ulceration, microscopic examination of the lesions revealed signs of healing vesiculation.

Discussion

Results of our study indicated that pigs can be experimentally infected with the 1995 equine isolate of VSV-NJ (CO) and the 1997 equine isolate of VSV-I (NM). However, viral shedding, development of vesicular lesions, and transmission of virus via contact were most evident in pigs infected with VSV-NJ (CO). Results of infection of pigs with VSV-NJ (CO) were very similar to results observed with VSV-NJ (OS) in the study of this report and previous studies[17-20]; pigs could be infected with both strains of VSV-NJ via ID inoculation of the snout, application of virus to scarified areas of the oral mucosa, and application of virus to intact oral mucosa. Pigs could be infected with VSV-NJ (CO) or VSV-NJ (OS) with an inoculum as small as 10^2 $TCID_{50}$ via ID inoculation of the snout, but lesions were produced only with viral doses ≥ $10^{3.6}$ $TCID_{50}$. Vesicular lesions were not observed in pigs that were infected with VSV-NJ (OS) at a dose of $10^{3.3}$ $TCID_{50}$ via ID inoculation of the snout. To date, vesicular lesions in pigs associated with this strain have only been reported at a dose ≥ 10^4 $TCID_{50}$.[19,23] As determined for VSV-NJ (OS) in our study and other investigations,[17-20] infection with VSV-NJ (CO) resulted in shedding as early as PID 1, viral shedding for as many as 6 consecutive days, and virus isolations from multiple swab samples collected from pigs with and without vesicular lesions. In the study of this report, fecal shedding of VSV-NJ (CO) was detected, which is similar to findings of a study[19] of infection with VSV-NJ (OS) in pigs. Consistent with the results of that study[19] of VSV-NJ (OS) infection, the viral concentration associated with the single fecal swab that yielded positive results in the study of this report was low (< $10^{2.3}$ $TCID_{50}$), shedding was observed on a single day, and the pig had vesicular lesions in the oral cavity.

In the study of this report, patterns of contact transmission of VSV-NJ (CO) in pigs also were consistent with patterns detected with VSV-NJ (OS) in our study and another investigation.[20] However, with regard to potential for contact transmission of VSV-NJ (CO), some differences may be associated with location of lesions (ie, on the snout vs in the oral cavity). Transmission of VSV-NJ (CO) was detected in only 2 of 12 pigs housed in contact with pigs that received ID inoculation of the snout, whereas transmission of the virus was detected in 8 of 12 pigs housed in contact with pigs that were inoculated via scarification of the oral mucosa. It is interesting that these results are consistent with virus isolation results from experiment 1 of our study; in that experiment, virus titers associated with pigs inoculated with VSV-NJ (CO) via scarified areas of oral mucosa exceeded those associated with pigs receiving ID inoculation of the snout. Such differences were not apparent in pigs inoculated with VSV-NJ (OS) in our study or other investigations.[18-20]

In our study, data obtained for the VSV-I (NM)-inoculated pigs were similar to but not consistent with data obtained for pigs inoculated with the VSV-NJ strains. As determined for the VSV-NJ strains, infection with VSV-I (NM) and resultant vesicular lesions were experimentally produced in pigs via ID inoculation of the snout and application of the virus to scarified areas of oral mucosa. The amount of virus required to produce these lesions ($10^{3.6}$ TCID^{50} for ID inoculation of the snout) mirrored results obtained for VSV-NJ strains in our study and other investigations.[17-19] As with the VSV-NJ strains, VSV-I (NM) also was recovered from multiple swab samples. However, unlike VSV-NJ strains, VSV-I (NM) concentrations associated with these swabs were low and exceeded a minimum detectable limit of $10^{2.3}$ $TCID_{50}$ in only 1 pig ($10^{3.6}$ $TCID_{50}$ from a single nasal swab). In addition, the duration of viral shedding in pigs inoculated with VSV-I (NM) was decreased (1 to 3 days), compared with that associated with pigs inoculated with VSV-NJ strains. These observations and the fact that pigs could not be infected via ID inoculation with the minimum viral dose of $10^{1.9}$ $TCID_{50}$ used in the study of this

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report are consistent with the low rate of contact transmission observed with this virus. This variation was not related to reduced virulence of the VSV-I (NM) isolate because, in the experience of one of the authors, inoculation of horses with Vero cell-propagated virus (obtained from the same stock samples used to produce the inocula used in our study) resulted in notable lesions and viral shedding comparable to that associated with inoculation of VSV-NJ.

We did not observe differences in response of pigs associated with inoculations of the 2 VSV-NJ isolates used in our study, but the possibility of variation between VSV-NJ strains related to virulence or host adaptation cannot be discounted and deserves further study. Although these viruses have considerable genetic variation over their geographic range[22,23] and VSV-NJ strain-related variation in clinical signs of infection has been reported,[24,25] the question of potential strain-related variation within specific domestic animal hosts has received little attention. Studies in domestic pigs may be an efficient means by which to initially address this question.

In addition to providing insight into potential variation in host response, results of the experimental infections of pigs investigated in the study of this report may also offer some insight into potential routes of transmission during outbreaks, with relevance to disease management. For example, viremia associated with infection with VSV-NJ or VSV-I in pigs or other domestic animal species has not been detected, and results of our study were consistent with this finding. Although this negates the possibility of infection of vectors through ingestion of blood, it does not indicate that insects cannot become infected through ingestion of virus associated with lesion material or skin or via cofeeding (ie, vector to vector transmission on a nonviremic host), as indicated by results of a study[26] in mice. These potential transmission mechanisms need to be defined if the potential role of numerous insect vectors in the epidemiology of these viruses is to be understood. Because of their size, housing requirements, and the reproducibility of experimentally induced clinical and subclinical vesicular stomatitis in domestic pigs, this species may represent an ideal model system for such studies. In addition, results of our study and those of other investigations are consistent with field observations. For example, results of the study of this report suggest that contact transmission would be much more likely with VSV-NJ than VSV-I; it is interesting that this apparent difference is consistent with results from field studies in areas of Central America where both VSV-NJ and VSV-I are enzootic. In cattle in Central America, a consistently higher prevalence of antibodies against VSV-NJ, compared with antibodies against VSV-I, has been reported; however, at the herd level (prevalence of seropositive herds), these differences were not detected.[16,27,28] These field results might be expected from assessment of the results of the study of this report; overall, it suggests that VSV-NJ has a much higher potential for transmission (probably through contact) once introduced into a domestic animal herd or population. If so, this would imply that control and eradication strategies for vesicular stomatitis may differ with regard to the causative viral strain (eg, VSV-NJ or VSV-I), despite the fact that the disease produced by these strains is clinically indistinguishable.

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[a]Provided by the National Veterinary Services Laboratory, Ames, Iowa (NVSL accession #95-44625).

[b]Provided by the National Veterinary Services Laboratory, Ames, Iowa (NVSL accession #97-25323).

[c]Sigma Chemical Co, St Louis, Mo.

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