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VENEREAL TRANSMISSION OF PSEUDORABIES VIRUSES INDIGENOUS TO FERAL SWINE

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ABSTRACT: Between 1995 and 1998, we designed a series of studies in which we attempted to determine the main routes of transmission involved in the natural infection of pseudorabies virus (PRV) indigenous to free-ranging feral swine (*Sus scrofa*). Naturally infected feral sows transmitted the infection to uninfected feral boars, with which they had been commingled for a 6-wk period. Pseudorabies virus was isolated from boar preputial swabs, but not from nasal swabs. Three of the same PRV-infected feral sows did not transmit the infection to domestic boars during a 16 wk commingling period, despite the fact that they became pregnant. Feral boars, naturally infected with PRV, transmitted the virus to domestic gilts while penned together during 6 wk. Pseudorabies virus was isolated from vaginal swabs, but not from nasal swabs of gilts, after 2 and 3 wk of commingling. When the same infected boars were commingled with either feral or domestic boars for 13 wk, PRV transmission did not occur. None of the exposed boars developed neutralizing antibodies or yielded virus from their preputial or nasal swabs. Our results indicate that PRV indigenous to feral swine is preferentially transmitted to feral or domestic swine of the opposite sex by the venereal route. This mode of transmission differs from that seen in the natural transmission of PRV prevalent in domestic swine, where contaminated secretions, excretions and aerosols are responsible for the spread of the virus. Based on these results, we feel that as long as feral swine do not come into direct contact with domestic swine, PRV-infected feral swine probably pose only a limited risk to the success of the National Pseudorabies Eradication Program. The fact that PRV is usually transmitted from feral to domestic swine at the time of mating would indicate that the isolation of domestic herds by the use of a "double fence," should be adequate protection against reinfection with PRV.

Key words: Feral swine, natural transmission, pigs, pseudorabies virus, seroconversion, *Sus scrofa,* veneral transmission, virus isolation.

INTRODUCTION

Pseudorabies, also known as Aujeszky's disease, is a costly disease for the swine industry worldwide. The disease is caused by an alpha herpesvirus and affects pigs of all ages. Economic losses for producers are due to high mortality in naïve newborn piglets, respiratory disease in both young and adult pigs, and abortion in pregnant sows (Kluge et al., 1999). The swine industry in these countries also suffers economically in the world market where sanitary trade barriers, with regard to PRV infection, are imposed. Countries that produce significant numbers of swine, such as the UK (Taylor, 1989) and Denmark (Anderson et al., 1989), have been free of the disease for a number of years. In 1989, the National Pseudorabies Eradication Program was initiated in this country. The objective of this program is to eliminate PRV infection from the domestic swine herd by the end of the year 2000. The program is well underway and, as of June 30, 2000, domestic pig herds from 33 states were in the final stage of the program and considered PRV-free (Slack, 2000).

In the United States, there exists a large free-roaming feral swine population, known to be PRV-infected, and calculated at over two million animals distributed throughout at least 24 states (Gipson et al., 1998). This herd poses a major threat to the completion of the eradication program, and also to subsequent efforts to maintain the national domestic swine herd PRV-free, once the disease has been eradicated.

Pseudorabies is transmitted to suscep-

tible pigs within domestic herds either by aerosols arising predominantly from the respiratory tract of infected pigs, or through exposure to virus contained in their secretions and excretions (McFerran and Dow, 1964). Wind-borne transmission across long distances is also known to have occurred, due to aerosolized virus that originated on infected premises (Christensen et al., 1993). Natural PRV infection of free-roaming feral swine (*Sus scrofa*) has been demonstrated in the United States (Nettles and Erikson, 1984; Van'der Leek et al., 1993) and in Europe (Oslage et al., 1994), by the presence of serum antibodies to PRV. More recently, direct evidence of PRV infection in free-roaming feral swine was obtained by isolating PRV from both oropharyngeal and genital swabs of feral boars and sows after immunosuppression with dexamethasone (Romero et al., 1997). Little is known about the mechanisms that operate in the natural transmission of PRV indigenous to feral swine. However, isolation of PRV from the genital and respiratory tract of immunosuppressed feral swine (Romero et al., 1997), and from the respiratory and genital tracts of experimentally infected swine or swine that had consumed infected carcasses (Hahn et al., 1997), indicate various routes of transmission. In this manuscript, we report on the venereal transmission of indigenous feral swine PRV to both feral and domestic pigs.

MATERIALS AND METHODS

Animals and housing

Feral swine utilized in these experiments were trapped and maintained at the Buck Island Ranch, MacArthur Agro-Ecology Research Center (Lake Placid, Florida, USA; 27°10′N, 81°21′W), between 1995 and 1998. Pigs were ear-tagged and a small blood sample was tested immediately for antibodies to both PRV (PRV Agglutination Test Kit, Viral Antigens, Inc., Memphis, Tennessee, USA), and *Brucella sp.* (*Brucella* Card Test, Becton-Dickinson Co., Inc., Baltimore, Maryland, USA). Animals were treated for gastrointestinal nematodes with Ivermectin (Meriel, Rahway, New Jersey, USA) and separated by both sex and PRV antibody status. Only animals testing negative for antibodies to *Brucella* were used for experimentation. Domestic boars and gilts were purchased from herds certified to be PRV- and *Brucella*-free. These animals were tested for the absence of PRV neutralizing antibodies before they were utilized in the various experiments. Commingling experiments were performed outdoors in two wooden slat pens, measuring 5 × 10 m, with dirt floors, food troughs and a continuous source of water. There was no roof over the pens, but nearby trees provided shade. Animals were fed a diet consisting mainly of corn and feed pellets once daily.

Virus isolation

Genital and nasal secretions for virus isolation were collected with sterile, Dacron swabs (Curtin Matheson Scientific, Houston, Texas, USA) and immediately placed into tubes with 2 ml of transport medium [Dulbecco's modified Eagle medium (DMEM); 4% fetal bovine serum (FBS); penicillin (300 U/ml); streptomycin (300 μg/ml); amphotericin B (25 μg/ml) and gentamycin (10 μg/ml) (Life Technologies, Grand Island, New York, USA)]. The swabs were frozen in dry ice and transported to the laboratory. For virus isolation, African green monkey kidney (Vero) cells (American Type Culture Collection, Manassas, Virginia, USA) were grown in DMEM, supplemented with 4% FBS and antibiotics, in 35 mm petri dishes. For the virus isolation assay, the tubes with swabs and transport medium were thawed, vortexed briefly, and the medium expressed from each swab by pressing against the side of the tube. The swab was then discarded. The medium was passed through a 0.45 μm syringe filter (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and 0.5 ml were inoculated directly onto drained Vero cell monolayers. After two hours of incubation, the inocula were removed and replaced with DMEM supplemented with 2% FBS and antibiotics. Cultures were monitored daily, for up to 10 days, for evidence of cytopathogenic changes characteristic of herpesviruses. Cultures were maintained as needed. Those cultures showing signs of herpesvirus infection were harvested and stored at −70 C. To determine the amount of infectious virus recovered per swab, fluids positive for PRV were further titrated in Vero cells under agarose (Life Technologies, Grand Island, New York, USA). The viral titer was calculated in plaque forming units (pfu) per 2 ml of transport medium. The isolates were identified as PRV by virus neutralization using swine antiserum specific for PRV (Romero et al., 1997).

Serology

Serum samples used for the determination of serum neutralizing antibodies to PRV, were heat inactivated at 56 C for 45 min and then assayed, in duplicate, in a virus neutralization test with 100 median tissue culture infectious doses ($TCID_{50}$) of the reference Shope strain of PRV (Romero et al., 1997).

Experimental design

In experiment 1, six naturally infected PRV antibody-positive feral sows were placed in a pen with five PRV antibody-negative feral boars for 6 wk, after which the sows were removed to another pen. The boars had tested serologically negative for PRV-neutralizing antibodies. Sow and boar nasal and genital swabs were negative for virus during the 4 wk prior to commingling. Nasal and genital swabs and blood samples were obtained from all animals at 0, 1, 2, 3, 4, 6, 8 and 12 wk.

For experiment 2, six naturally infected PRV antibody-positive feral boars were placed in a pen for 6 wk with seven domestic gilts. The boars were then removed to another pen. The gilts had tested negative for PRV neutralizing antibodies 1 wk before they were commingled with the infected feral boars. Nasal and genital swabs from all animals tested negative for virus 1 wk prior to commingling. Nasal and genital swabs and blood samples were obtained from all animals at 0, 1, 2, 3, 4, 6, 8, 10 and 12 wk.

In experiment 3, four feral boars that had nasal and preputial swabs negative for PRV isolation, and sera that were negative for neutralizing PRV antibodies for 4 wk, were commingled with six naturally PRV-infected feral boars. The animals remained together during 13 wk, at which time the infected boars were removed from the pen. Nasal and preputial swabs, as well as blood samples, were obtained from all animals at 0, 1, 2, 3, 4, 6, 9, 13, 18 and 20 wk.

For experiment 4, six naturally PRV-infected feral boars were commingled in a pen for 13 wk with six PRV-negative domestic boars. The feral boars were removed to another pen after 13 wk. Nasal and preputial swabs and blood samples were obtained from all animals at 0, 1, 2, 4, 6, 8, 12, 13, 18 and 20 wk.

In experiment 5, three naturally PRV-infected feral sows were placed in a pen for 16 wk with three domestic boars obtained from a certified PRV-free herd. Nasal and genital swabs and blood samples were obtained from all pigs at 0, 1, 2, 4, 7, 10, 12 and 16 wk.

RESULTS

In experiment 1, where naturally PRV-infected feral sows were commingled with uninfected feral boars for 6 wk, evidence of PRV transmission was demonstrated when PRV was isolated from preputial swabs from three of the boars. After the animals had been penned together for only 1 wk, PRV was recovered from a preputial swab from one of the boars. Further evidence of venereal transmission was obtained at wk 2 (three isolates) and wk 3 (one isolate). Viral titrations showed that the positive swabs contained between 68 and 176 pfu at 2 wk and 20 pfu at 3 wk. Pseudorabies virus was not isolated from the vaginal swabs from PRV-infected feral sows. Pseudorabies virus infection was also confirmed by the demonstration of serum neutralizing antibodies in the same three boars 2 wk after commingling (Table 1).

For experiment 2, naturally PRV-infected feral boars were commingled with uninfected domestic gilts during 6 wk. Evidence of venereal transmission was first obtained 2 wk after commingling, when PRV was isolated from vaginal swabs from two gilts. Pseudorabies virus was also recovered from the vaginal swabs of one of these gilts at 3 wk. The quantity of virus contained in individual swabs varied between 106 and 240 pfu. Pseudorabies virus was not recovered from the preputial swabs taken from the PRV-infected boars during the commingling period. Furthermore, PRV transmission from the naturally infected feral boars to six of the seven PRV-negative domestic gilts was confirmed by the demonstration of serum neutralizing antibodies in three of the gilts at wk 3, and in six gilts at wk 4 (Table 2). However, in three of the six antibody-positive gilts, neutralizing titers were transient in nature and had disappeared by wk 10. One of the gilts never developed serum neutralizing antibodies to PRV.

Experiment 3 involved placing six naturally PRV-infected feral boars together with four uninfected feral boars for 13 wk. Four of the infected boars had been previously used in experiment 2. Pseudorabies virus was never isolated from any of the preputial swabs from infected or uninfect-

TABLE 1. Development of serum neutralizing antibodies to pseudorabies virus (PRV) after uninfected feral boars were commingled with naturally PRV-infected feral sows for 6 wk.

		Weeks									
Number		−4	−3	0[a]	1	2	3	4	6[b]	8	12
Sow	44	24[c]	32	16	32	16	48	64	64	64	32
	168	64	96	64	64	32	128	192	96	96	64
	170	32	48	128	32	64	64	48	192	Nd[d]	Nd
	172	16	128	32	64	64	128	48	128	96	96
	417	4	8	8	32	8	48	32	48	32	16
	424	8	32	16	4	16	8	48	48	48	16
Boar	164	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
	193	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
	196	<2	<2	<2	<2	8	48	24	12	32	32
	198	<2	<2	<2	<2	2	32	32	48	32	Nd
	199	<2	<2	<2	<2	8	12	8	4	48	Nd

[a] Beginning of commingling period.
[b] Sows removed to another pen.
[c] Reciprocal of the highest serum dilution (mean of duplicates) that neutralized 100 median tissue culture infectious doses of the Shope strain of pseudorabies virus.
[d] Nd = not done.

ed feral boars (data not shown). Similarly, the uninfected feral boars did not develop PRV neutralizing antibodies during the experimental period of 20 wk (Table 3).

In experiment 4, six naturally PRV-infected feral boars were commingled for 13 wk with six uninfected domestic boars. Pseudorabies virus was not isolated from preputial swabs from feral or domestic boars during the 13-wk experimental period (data not shown). Furthermore, none of the six PRV-negative domestic boars developed neutralizing antibodies through 20 wk of testing (Table 4).

Experiment 5 penned three naturally PRV-infected feral sows, all of which had

TABLE 2. Development of serum neutralizing antibodies to pseudorabies virus (PRV) after domestic gilts were commingled with naturally PRV-infected feral boars for 6 wk.

		Weeks									
Number		−1	0[a]	1	2	3	4	6[b]	8	10	12
Boar	36	Nd[c]	96[d]	48	128	32	48	64	Nd	Nd	Nd
	39	Nd	48	64	64	32	32	Nd	Nd	Nd	Nd
	166	Nd	48	48	48	96	64	48	Nd	Nd	Nd
	196	Nd	24	16	32	24	24	24	Nd	Nd	Nd
	388	Nd	32	16	24	48	32	32	Nd	Nd	Nd
	389	Nd	64	96	64	96	64	48	Nd	Nd	Nd
Gilt	105	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
	106	<2	<2	<2	<2	8	16	8	4	4	4
	107	<2	<2	<2	<2	8	64	64	12	8	12
	108	<2	<2	<2	<2	8	16	12	2	<2	2
	109	<2	<2	<2	<2	<2	6	8	4	4	8
	110	<2	<2	<2	<2	<2	8	4	<2	<2	<2
	111	<2	<2	<2	<2	<2	3	16	<2	<2	<2

[a] Beginning of commingling period.
[b] Boars removed to another pen.
[c] Nd = not done.
[d] Reciprocal of the highest serum dilution (mean of duplicates) that neutralized 100 median tissue culture infectious doses of the Shope strain of pseudorabies virus.

TABLE 3. Serum neutralizing antibodies to pseudorabies virus (PRV) after naturally PRV-infected feral boars were commingled with uninfected feral boars for 13 wk.

	Weeks										
Boar number	−4	0[a]	1	2	3	4	6	9	13[b]	18	20
Infected											
36	Nd[c]	64[d]	64	64	128	128	64	64	64	Nd	64
39	Nd	32	32	32	64	128	32	32	64	Nd	64
267	Nd	64	64	64	64	64	32	48	64	Nd	Nd
276	Nd	64	64	128	64	128	64	96	64	Nd	Nd
388	Nd	32	16	32	32	64	16	64	64	Nd	48
389	Nd	32	32	64	64	64	64	64	64	Nd	96
Uninfected											
193	<2	<2	<2	2	2	<2	<2	2	2	<2	<2
196	<2	<2	<2	<2	2	<2	<2	2	<2	<2	<2
198	<2	<2	<2	2	<2	<2	<2	<2	<2	<2	<2
199	<2	2	<2	<2	<2	<2	<2	<2	<2	<2	<2

[a] Beginning of commingling period.
[b] Infected boars removed to another pen.
[c] Nd = not done.
[d] Reciprocal of the highest serum dilution (mean of duplicates) that neutralized 100 median tissue culture infectious doses of the Shope strain of pseudorabies virus.

been used in experiment 1, together with three PRV-free domestic boars during 16 wk. Pseudorabies virus was not isolated from any of the genital swabs taken from the experimental animals and none of the domestic boars developed neutralizing antibodies during the 16-wk experimental period (Table 5).

Pseudorabies virus was never isolated from nasal swabs, at any time, during any of the five experiments.

DISCUSSION

Herein, we provide evidence for venereal transmission as the most important route of natural transmission of pseudorabies viruses indigenous to feral swine, in both free-ranging feral and domestic swine. In earlier studies on an isolated population of feral swine in Ossabaw Island (Georgia, USA) Pirtle et al. (1989) showed that antibodies to PRV occurred primarily in adult feral swine, with little evidence of seroconversion or maternal antibodies in juvenile feral swine. Preliminary evidence that venereal transmission might be the preferential route of PRV transmission among free-ranging feral swine was obtained when PRV was isolated from genital swabs of dexamethasone-treated PRV antibody-positive feral swine (Romero et al., 1997). More recently, PRV was isolated from a vaginal swab from one feral sow and from preputial swabs from five feral boars trapped at Ossabaw Island (D. Stallknecht, pers. comm.). Nasal swabs from the same animals were all virus negative.

Both feral and domestic swine are susceptible to infection by feral swine PRV by different routes, evidence of which has been demonstrated experimentally by either feeding infected pig carcasses or by applying virus directly into the upper respiratory passages (Hahn et al., 1997).

None of the feral swine or domestic pigs that acquired the infection showed any overt clinical signs characteristic of pseudorabies infection. These observations support previous findings that PRV isolated from feral swine behave like attenuated domestic PRV strains when inoculated into domestic swine (Hahn et al., 1997). This has prompted us to speculate that, if indigenous feral swine PRV were transmitted to domestic swine, any symptoms of the infection would be subclinical and, most likely, go unnoticed.

TABLE 4. Serum neutralizing antibodies to pseudorabies virus (PRV) after naturally PRV-infected feral boars were commingled with uninfected domestic boars for 13 wk.

	Weeks								
Boar Number	0[a]	1	2	4	6	8	12	13[b]	20
Feral									
36	64[c]	64	128	48	128	48	Nd[d]	Nd	Nd
39	Nd	Nd	32	128	32	48	Nd	Nd	Nd
166	48	48	48	48	Nd	24	64	Nd	Nd
196	24	16	32	16	24	12	32	Nd	Nd
388	32	24	64	24	24	16	16	Nd	Nd
389	48	32	64	48	96	24	32	Nd	Nd
Domestic									
127	<2	<2	<2	<2	<2	<2	<2	<2	<2
128	<2	<2	<2	<2	<2	<2	<2	<2	<2
129	<2	<2	<2	<2	<2	<2	<2	<2	<2
130	<2	<2	<2	<2	<2	<2	<2	<2	<2
131	<2	<2	<2	<2	<2	<2	<2	<2	<2
132	<2	<2	<2	<2	<2	<2	<2	<2	<2

[a] Beginning of commingling period.
[b] Feral boars removed to another pen.
[c] Reciprocal of the highest serum dilution (mean of duplicates) that neutralized 100 median tissue culture infectious doses of the Shope strain of pseudorabies virus.
[d] Nd = not done.

Sexually active uninfected feral boars of experiment 1 were rapidly infected when allowed to commingle with infected feral sows, as evidenced by the isolation of infectious PRV from preputial secretions 1 wk after commingling. These boars also developed PRV-neutralizing antibodies (Table 1). Alternatively, infectious PRV was not isolated from the nasal swabs taken from the same boars. It was also very interesting to note, that at the time these infected feral sows were transmitting PRV to the feral boars, PRV could not be isolated from either their vaginal or nasal swabs. These findings may indicate that either the boars were under higher stress, associated with the presence of the sows, or that the boar genital tract has a higher level of susceptibility to PRV infection and produces more virus than the genital tract of the sow.

The fact that feral boars are extremely

TABLE 5. Serum neutralizing antibodies to pseudorabies virus (PRV) after naturally PRV-infected feral sows were commingled with uninfected domestic boars for 16 wk.

	Weeks							
Swine number	0[a]	1	2	4	7	10	12	16
Feral								
170	Nd[b]	96[c]	96	64	64	96	128	96
417	Nd	12	32	24	32	48	48	48
424	Nd	32	16	12	24	32	24	12
Domestic								
127	<2	<2	2	<2	<2	2	<2	<2
130	<2	<2	<2	<2	<2	<2	<2	<2
131	<2	<2	<2	<2	<2	<2	<2	<2

[a] Beginning of commingling period.
[b] Nd = not done.
[c] Reciprocal of the highest serum dilution (mean of duplicates) that neutralized 100 median tissue culture infectious doses of the Shope strain of pseudorabies virus.

sexually active animals, not easily discouraged by even the most non-receptive of feral sows, may be the most important single factor in the spread of PRV in free-ranging feral swine. This accentuated sexual behavior was also apparent when infected feral boars were commingled with domestic gilts. The gilts of experiment 2 were very rapidly mated and infected, a fact that was demonstrated by PRV recovery from their vaginal secretions and the subsequent development of PRV-specific neutralizing antibodies (Table 2). The transient nature of the neutralizing antibody titers observed in the sera of some of the gilts, may indicate that the indigenous PRV of feral swine were of low virulence.

Domestic boars did not exhibit the same aggressive sexual behavior of the feral boars when they were commingled with PRV-infected feral sows. Although all three feral sows in experiment 5 became pregnant sometime during the 16 wk of commingling, PRV was not transmitted from the infected feral sows to the domestic boars (Table 5). Most likely, the domestic boars did not repeatedly mate with the feral sows, and the reduced sexual contact may have contributed to the failure of PRV transmission.

Further support for our hypothesis that the preferential mode of PRV transmission is by the venereal route, was provided by the lack of transmission of the virus by infected feral boars to uninfected feral boars (Table 3) or domestic boars (Table 4), penned together for 13 wk. In a previous study, PRV-infected feral boars had transmitted the virus to feral sows by this route (Romero et al., 1997).

We have concluded from this set of experiments that indigenous feral swine PRV can be transmitted under conditions of intense, direct sexual contact. This is especially true in the case of the more sexually aggressive feral boar, known to mate frequently and with more than one sow. Conversely, PRV was not transmitted when feral swine were kept under identical conditions with swine of the same sex, convincing us that the main mode of transmission of indigenous feral swine PRV is not by the respiratory route, the traditional route for transmission of domestic swine PRV strains (McFerran and Dow, 1964).

The implications of these findings for the National Pseudorabies Eradication Program are profound. Feral swine, infected with indigenous PRV, are unlikely to transmit the virus to domestic pigs by the aerosol route. This being the case, the use of a "double fence" to physically isolate pseudorabies-free, domestic swine herds, should be sufficient to protect these animals from infection with feral swine PRV. In order for PRV-infected free-ranging feral swine to have the opportunity to infect PRV-free domestic herds, there would have to be a breakdown of the physical barriers and sexual contact.

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