RESEARCH REPORT

SWINE HEALTH

Title: Infection of domestic pigs with pseudorabies virus isolated from feral pigs.
NPB # 98-055

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I. Abstract:

Many feral swine populations are infected with pseudorabies virus and thus represent a significant reservoir for pseudorabies virus. Infections have been confirmed through virus isolations from the genital tract of naturally infected feral swine. However, unlike domestic swine where oronasal transmission primarily occurs, transmission is believed to be venereal in feral swine herds. Research is needed to understand the risk that feral swine represent to the maintenance of pseudorabies-free domestic swine. The objectives of this project were to determine if domestic pigs can be infected with feral swine strains of pseudorabies virus via the genital and respiratory routes and to determine the extent and sites of virus shedding and latency.

We inoculated gilts and boars with a strain of pseudorabies virus that was isolated from the prepuce of a feral swine. Virus was either inoculated into the nasal cavity or into the genital tract (vagina for gilts and prepuce for boars). We found that this virus could infect both boars and gilts when inoculated by either route and that virus was primarily shed from the area of inoculation. Although the virus was difficult to reactive by steroid treatment, it did appear to colonize the central nervous system with the sites of latency dependent on route of inoculation. We have shown that strains of pseudorabies virus isolated from the genital tract of feral swine have the potential to spread to domestic swine by either venereal or oronasal transmission and that once introduced latent infections could develop.

II. Introduction:

Many feral pig populations in the United States are infected with pseudorabies virus. Although the National Pseudorabies Virus Eradication Program will probably be successful in eliminating infection from domestic swine in the near future, feral swine could be a source of virus re-introduction into domestic herds. Such a real or perceived

risk could severely affect international trade. The results of this study have immediate application to understanding the risk of pseudorabies virus transmission from feral to domestic swine and the risk of persistence within a domestic swine herd should such an introduction occur.

III. Objectives:

The goal of this project was to determine if a "wild type" pseudorabies virus associated with venereal transmission in feral swine would demonstrate a tropism for the genital tract of domestic swine or if the course of infection would be determined by the route of transmission. Specific objectives were: (1) to determine if domestic gilts and boars could be infected with this virus via the genital and respiratory routes and (2) to determine the extent and routes of viral shedding and virus latency.

IV. Procedures:

Eight breeding age gilts and 8 breeding age boars were inoculated with an isolate of pseudorabies virus which was isolated from the prepuce of an adult feral pig. Four gilts and 5 boars were inoculated intra-nasally with approximately 10^{5-6} $TCID_{50}$ of PRV and 4 gilts and 4 boars were inoculated intra-vaginally or intra-preputially, respectively, with approximately 10^{5-6} $TCID_{50}$ of PRV. Gilts were inoculated during estrus that had been hormonally induced. Animals were evaluated for virus shedding from nasal cavity, tonsil, and genital tract (vagina or prepuce) from post inoculation day 0 until shedding ceased. Animals were tested for seroconversion to pseudorabies virus at 14 days post inoculation. Forty days post inoculation animals received dexamethasone for 6 consecutive days by a combination of intra-muscular and oral administration in an attempt to reactivate the virus. Animals were evaluated for virus shedding from post-steroid treatment day 0 to post-steroid treatment day 10 and then killed and necropsied. At necropsy a variety of tissues were sampled for immunohistochemistry, in situ hybridization, and polymerase chain reaction (PCR) to establish possible sites of latency.

V. Results:

Objective 1: Gilts and boars became infected with this strain of pseudorabies by both the nasal or genital routes as determined by viral shedding and seroconversion (Table 1). All gilts inoculated became infected. All boars inoculated intra-nasally became infected and 3 of 4 inoculated intra-preputially became infected. Clinical signs were limited. Some animals inoculated via the genital tract had vague ataxia in the rear during the first few weeks post inoculation. One boar inoculated intra-nasally was listless and vomited for 2 days during the initial infection.

TABLE 1. Number of animals infected

	Nasal inoculation	Genital inoculation
Gilts	4/4 (100%)*	4/4 (100%)
Boars	4/4 (100%)	3/4 (75%)

*Number of animals infected/number of animals inoculated

Objective 2:

During the first two weeks following inoculation, all gilts shed virus from the site of inoculation at some point in time (Table 2). Tonsillar shedding was more likely to occur in animals inoculated intra-nasally. Animals inoculated intra-nasally never

shed from the vagina and animals inoculated into vagina never shed from the nasal cavity. Virus shedding from the site of inoculation began post inoculation day (PID) 1-2, typically occurred for many days, but had ended by PID 15 (Figs. 1&2). Titers were relatively high. Titers recovered from tonsil were relatively low, except in one gilt that was inoculated intra-vaginally (Fig. 3). None of the gilts shed virus detectable by virus isolation following steroid treatment to reactivate the virus.

In the first two weeks following inoculation, all boars that became infected shed virus from the site of inoculation at some point in time (TABLE 2). Tonsillar shedding occurred in both groups, but was more likely to occur in animals inoculated intra-nasally. Although shedding was most likely to occur from site of inoculation, 2 boars inoculated intra-nasally shed virus from the prepuce and 1 boar inoculated intra-preputially shed virus from the nasal cavity. Thus, extent of virus shedding appeared to be greater in the boars than gilts. Virus shedding from the nasal cavity first occurred on PID 1-2 in intra-nasally inoculated boars. Virus shedding from the prepuce first occurred on PID 2-4 in intra-preputially inoculated boars. Duration of shedding from the various sites is presented in Table 3. Like the gilts, none of the boars shed virus detectable by virus isolation following steroid treatment to reactivate the virus.

TABLE 2. Virus shedding from three sites during first two weeks following inoculation

	Tonsil[#]	Nasal	Genital
Gilts-Intra-nasal[+]	4/4*	4/4	0/4
Gilts-Intra-vaginal	1/4	0/4	4/4
Boars-Intra-nasal	4/4	4/4	2/4
Boars-Intra-preputial	2/3	1/3	3/3

[#]Site of virus shedding. Genital= vagina or prepuce.
[*]Number of animals that shed virus/number of animals infected.
[+]Sex and route of inoculation.

TABLE 3. Duration of viral shedding in boars during first 2 weeks of infection.

	Prepuce[#]	Nasal	Tonsil
Intra-nasal inoculation	1-2*	7-9	4-7
Intra-preputial inoculation	4-9	1	2-5

[#]Site of inoculation
*Duration in days

Polymerase chain reaction, immunohistochemistry, and in situ hybridization results indicated that latency developed, but extent and site were dependent on route of inoculation. Polymerase chain reaction results from the gilts (Table 4) indicated that intranasal inoculation was followed by latent infection of the brain stem, trigeminal ganglia, and tonsil. Intravaginal inoculation resulted in less extensive colonization. Polymerase chain reaction results from boars are pending euthanasia of last group. Although polymerase chain reaction did not detect viral DNA in the sacral spinal cord of 4 of the infected gilts tested, immunohistochemistry and in situ hybridization showed virus antigen and DNA, respectively, in the sacral spinal cord and sacral ganglia of 1 of the intra-vaginally infected animals.

TABLE 4. Polymerase chain reaction results from gilts.

	Tonsil[#]	Trigeminal ganglia	Brain stem	Vagina	Sacral spinal cord	Lumbar spinal cord	Cervical spinal cord
Intra-nasal*	3/4[+]	4/4	4/4	0/2	0/2	0/2	0/2
Intra-vaginal	0/4	1/4	0/4	½	0/2	0/2	0/2

Site sampled
* Route of inoculation
+ Number of animals with positive polymerase chain results/number of infected animals tested

We have shown that a “wild type” pseudorabies virus, isolated from the prepuce of a feral pig, can infect domestic gilts and boars when inoculated by either respiratory or genital routes. Thus, the potential exists for re-introduction of pseudorabies virus into pseudorabies-free herds from feral swine if co-mingling of animals were to occur. Re-introduction would not be dependent on venereal transmission. Furthermore, we have shown that latency can develop with this isolate that there is risk of persistence of these feral swine isolates within domestic herds should re-introduction occur.

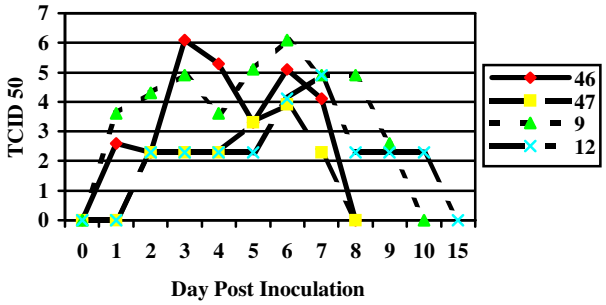

Fig. 1. Nasal Shedding in gilts- Intranasal Inoculation

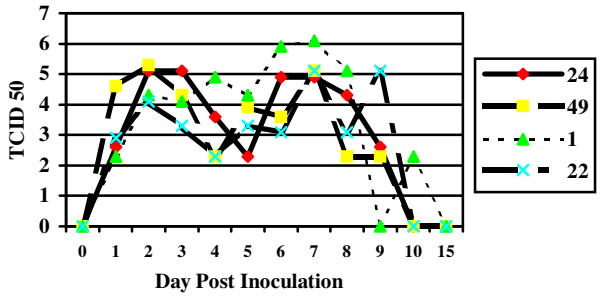

Fig. 2. Vaginal shedding in gilts- Intra-vaginal Inoculation

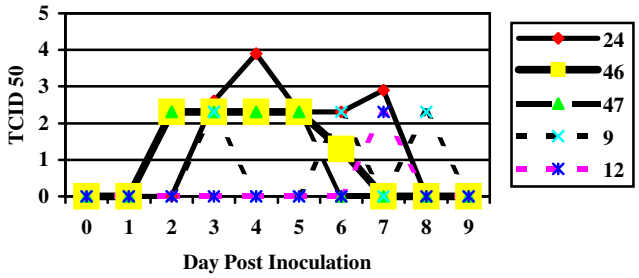

Fig. 3. Tonsillar Shedding in gilts