Evaluation of a TK, gpX, and gpI gene deleted pseudorabies vaccine

by

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EXPLANATION OF THESIS FORMAT

The following thesis is in the alternate format and consists of a general introduction, a review of the literature, two separate manuscripts (Sections I and II), a general summary and discussion, and literature cited in the literature review. Both manuscripts will be submitted for publication in the Journal of Veterinary Diagnostic Investigation. The master's candidate, Sabrina Lynn Swenson, is the senior author and principal investigator for each of the manuscripts.

GENERAL INTRODUCTION

Pseudorabies (PR) is a viral disease which affects many animal species and has the ability to cause a wide range of clinical signs, including death. This disease is the result of infection by pseudorabies virus (PRV), a herpesvirus. Pseudorabies is found worldwide, and is a disease of major economic importance in the swine industry. Factors such as type of operation, prevalence of PR, strain of PRV, and vaccination and treatment costs are important in determining the final cost of PR to the producer. It has been estimated that in a farrow-to-finish operation, the average cost of PR is $22.66/sow/year.[17] In farrow-to-finish herds in which PRV has severe effects on the pig population, the cost of PR to the producer has been estimated to be $74.75/sow/year.[17] In the last quarter of this century, many countries have taken steps towards eradicating PRV. In the United States, policies have been developed at both the state and federal level in order to halt the spread of PRV, and to eventually eradicate the virus from the country. Steps that have been taken include: testing of swine sera to detect the presence of PRV antibodies, restricted movement of infected swine, and the use of vaccines to reduce the severity of the clinical disease and to reduce viral excretion by infected pigs. In the last ten years, major technological advances have made it possible to develop gene deleted vaccines which are not only safe and efficacious, but which have also been designed to distinguish noninfected, vaccinated pigs from infected pigs through the use of special serological tests. With the use of these vaccines and companion diagnostic tests, as well as restricted swine movement, it is hoped that PRV can be eradicated from the United States.

LITERATURE REVIEW

Introduction

Pseudorabies virus is a herpesvirus which belongs in the family Herpesviridae and the subfamily Alphaherpesvirinae.[18] Pseudorabies is also known as mad itch, Aujeszky's disease, and infectious bulbar paralysis.[23] In 1902, Aujeszky gave the first scientific description of the disease caused by PRV.[3] It is not known how long PRV has been in the United States, but a reference from the early 1800's describes a disease of cattle characterized by excessive itching which may have been PR.[23]

Pseudorabies virus has the ability to infect a wide range of animals, including many of our domestic animals. Pseudorabies in cattle, sheep, dogs, and cats, results in a disease which rapidly progresses until death of the animal.[43] Swine are considered to be the primary reservoir of PRV and are capable of transmitting the virus to other host animals.[43]

Until the 1960's, PR in swine was considered to be primarily a disease of young pigs. In the early 1960's, a highly virulent form of PRV rapidly spread through Indiana and into surrounding states.[20,54] In California, there were reports of PRV infections in feeder pigs resulting in high mortality rates.[25] By the end of 1976, PRV infections were considered to be a significant problem for the swine industry in at least twenty-three states[5], and by 1979, the federal government had developed regulations to control the movement of feeder pigs and breeding stock.[10,11]

Pseudorabies virus enters the host through the oral and nasal routes.[11] The virus is transmitted from one pig to another via infectious respiratory

secretions.[11,20,34,47] This transmission may be from direct contact with infected pigs, or may be from contact with contaminated fomites[20], such as boots and bedding. Once in the host, the virus rapidly replicates in the nasal, oropharyngeal, and respiratory tissues.[18,43,55] Viremia may develop if the virus infects white blood cells.[55] Studies have shown that PRV has the ability to spread to various tissues in the body by migrating through the nervous system. Field and Hill[19] studied the spread of PRV in mice by inoculating the rear foot pad and then examining nervous tissue for the presence of PRV. They found that PRV spread from the foot to the brain via the sequential movement from the sciatic nerve to the dorsal root ganglia, and then to the spinal cord and brain. When the sciatic and femoral nerves were disrupted, mice infected with PRV developed less severe clinical signs compared to those with intact nerves. Additional studies have shown that PRV spreads from the upper respiratory tract and nasopharyngeal region to the brain via the olfactory, glossopharyngeal, facial, vagus, and trigeminal nerves.[36,43,48,55]

The disease caused by PRV is often age-dependent, with the youngest pigs having the most severe clinical signs and the highest mortality rates.[11,15,20,43] In young pigs, PRV infections are characterized by elevated temperatures, depression, anorexia, lethargy and central nervous system (CNS) disorders. Pseudorabies in pigs may result in muscle tremors, incoordination, paralysis, seizures, and sometimes culminates in death.[11,20] Those pigs that survive are often stunted and do not grow as rapidly as unaffected pigs. The disease in older swine is characterized by a febrile response, anorexia, depression, weight loss, and respiratory disorders such as nasal discharge, pneumonia, and dyspnea.[11,54] This age group of pigs can also develop CNS signs and may die,

however, the mortality rate is low. Breeding age pigs may have clinical signs similar to feeder pigs. The greatest economic loss in breeding age animals is due to reproductive disorders. Depending on the stage of gestation when the sow or gilt is infected, the female can have a variety of clinical signs. Infection during the early stages of gestation results in the death and resorption of the fetuses.[15,20,29,56] Infection later in gestation results in the birth of mummified fetuses, dead, weak, or clinically normal pigs.[15,20,29,56]

Pseudorabies Virus

Pseudorabies virus is a DNA virus with a diameter of approximately 150-180 nm.[11,18] The basic viral structure is a linear, double-stranded DNA core surrounded by a capsid and encased in a lipid and glycoprotein envelope.[18,56] The DNA can be divided into four functional regions: two inverted repeat sequences, a unique long sequence (U_L), and a unique short sequence (U_S).[6] The U_S is flanked by an internal and terminal inverted repeat sequence. The U_L is associated with the internal inverted repeat.[56] Several genes within the U_L and U_S code for glycoproteins (gp) which are found in the envelope of the virus. Due to their location in the virus, these gps play a role in the antigenicity of the virus, and a role in the attachment to, multiplication within, and release from a host cell.[7,8,31] Genes within the U_L and U_S also code for products which play a role in the virulence of the virus.[7,9,30,31,44] The complete function of the inverted repeats is not known, but they do play a role in the virulence of the virus.[9]

Three genes that are located in the U_L, and code for important proteins, are the gpII, gpIII, and thymidine kinase (TK) genes. Glycoprotein II and gpIII play

different roles in PRV function. Glycoprotein III is not essential for replication, but it it partly responsible for the virulence of the virus and allows for the virus to attach to a host cell.[56] Removal of this gene from the viral DNA reduces the virulence of the virus, but doesn't alter viral replication.[56] On the other hand, gpII can not be removed without altering replication of the virus.[56] Thymidine kinase is partly responsible for the degree of virulence of PRV.[31] This enzyme allows PRV to replicate within host neural tissue. Some of the clinical signs observed with PRV infections are due to the virus' ability to replicate within nervous tissue. When the TK gene is removed from the genome, the degree of viral virulence is reduced.[56]

Within the U_S, there are genes which code for several known gps. One of these gps, gp50, is a target of neutralizing antibodies and can not be removed from the virus without altering viral growth.[56] Glycoprotein I, gp63, and gpX are three additional gps that are produced by genes found in the U_S, and can be deleted from the genome without adverse effects on viral replication.[56] Like thymidine kinase, gpI plays an important role in the virulence of the virus.[7] In addition, gpI is also a minor target for neutralizing antibodies.[56] This gp can be deleted from the genome without seriously affecting the antigenicity of the virus.[29] The function of gp63 and gpX is unknown. In vitro experiments have shown that gpX is produced in high quantities and is released into the cell culture media.[8,50,56]

Two important aspects of PRV are that the degree of virulence of the virus is controlled by several genes,[31] and that the virus has the ability to latently infect a host.[18,20,56] Latently infected pigs which have recovered from an infection with PRV, have the ability to shed virus into the environment.

Studies have shown that stress such as fluctuating environmental temperatures, transportation, and doses of steroids such as dexamethasone have the ability to cause a pig to shed virus.[11,12,27,40,46] Latently infected pigs become a source of virus for susceptible pigs. In the carrier state, it is difficult to detect virus in tissues and secretions.[24,53] These pigs often do not shed virus into nasal and oral secretions, and virus may not be detectable in tonsils using standard fluorescent antibody and virus isolation procedures. Special techniques such as explantation[45] and RNA-DNA hybridization[21,22] have been used to identify latently infected pigs which are not shedding virus.

Vaccines

The first PR vaccines were licensed for use in the United States in 1977.[5,11] Vaccines for PR have been shown to reduce the degree of viral replication and spread throughout the body.[16,35] The net result is a decreased severity of disease in the infected pigs. Although the vaccines are able to reduce the clinical signs of PR, they are not able to prevent infection of pigs, and virus is still able to circulate through a herd.[1,16,32,38,40,49] Infected pigs will still shed virus into the environment, but they shed smaller amounts of virus and for fewer days compared to nonvaccinated pigs.[16,35,37,42] As a result of decreased secretion of virus, the chance of susceptible pigs becoming infected is reduced.

In recent years, there has been a trend towards developing vaccines that have gene deletions in order to be able to distinguish vaccinated pigs from infected pigs.[28,33,38] Gene deleted vaccines that have a companion diagnostic test and are currently licensed in the United States have deletions of gpI, gpIII, or gpX. Through the use of these differentiable vaccines, it has been easier to

develop herds free of PRV. Both killed and modified live virus (MLV) vaccines have been developed for use in swine. The two different types of vaccines are both safe and effective in reducing clinical disease, but have been developed and used for different reasons.

Killed vaccines

Killed vaccines are inactivated by using a variety of chemical agents such as ethylenimine and acetylethylenimine.[51] These vaccines are usually made by inactivating the entire virus particle.[38] Killed vaccines contain adjuvants to enhance the immune response to the PRV antigens. Hypersensitivity reactions and interference with the detection of contaminating agents are two problems that may arise from the use of adjuvants in vaccines.[37,38] In theory, killed vaccines are safer than MLV vaccines because live agents in the vaccine have been destroyed by the chemical agent.[37] This situation does not always hold true in the field situation. Microorganisms that are resistant to the chemical agent may contaminate the vaccine, resulting in the vaccination of pigs with live microorganisms. Although this does not occur very often, it is a potential disadvantage of killed vaccines. Detection of contaminating agents may be difficult due to the presence of the adjuvant in the vaccine.[37]

Killed vaccines are effective in stimulating the production of protective antibodies.[37] In most instances, it is preferred to give two doses in order to develop the best antibody response.[10,38,57] Due to the lack of viral replication with killed vaccines, there is usually a lower antibody titer, a shorter duration of immunity, and a reduced development of local antibodies (immunoglobulin A) at the site of entry in the oral and respiratory tissues.[38,41]

The development of a humoral immune response when killed vaccines are used is reduced in the presence of maternal antibodies.[13,52]

Modified live vaccines

Modified live virus PR vaccines also reduce the severity of PRV infections. An important aspect of MLV vaccines is that the virus undergoes replication in the pig.[41] This mimics the events that occur when a pig becomes infected with field strains of virus. With vaccine virus replication, there is an increase in the antigenic mass that the pig is exposed to at the site of inoculation and in the regional lymph nodes.[41] The net effects of viral replication are higher antibody titers with a single dose of vaccine and longer lasting immunity.[57] Like killed vaccines, the development of a humoral immune response is reduced in the presence of maternal antibodies.[13,52] This interference with antibody production can be reduced when MLV vaccines are given intranasally.[56]

Modified live virus vaccines have several potential disadvantages. Since these vaccines contain live virus, there is the potential for the virus to revert to a more virulent form.[37,38,43] If this occurs, vaccinated pigs may develop clinical disease. Also, other viral or bacterial agents may contaminate the vaccine in the manufacturing process.[38] Another problem which may arise with the use of MLV vaccines is the potential for vaccine virus to be transmitted to pigs that are in close contact with the vaccinated pig.[37,43] The spread of vaccine virus to non-vaccinated pigs does not appear to be a significant problem as many researchers have found that viral excretion does not occur post-vaccination.[2,4,13,26,35]

Section I. EVALUATION OF THE SAFETY AND EFFICACY OF A TK, GPX, AND GI DELETED PSEUDORABIES VACCINE

SUMMARY

The experimental modified live pseudorabies vaccine SV/PRV[a] was evaluated for both safety in swine and for its efficacy in protecting swine against challenge with pseudorabies virus (PRV). Safety was evaluated by inoculating pregnant gilts intravenously with a standard 2 ml field dose of SV/PRV. Litters were examined at the time of parturition and the number of dead pigs was recorded. Safety was also evaluated by inoculating 3 day old pigs intracerebrally with a standard field dose in a 0.2 ml volume. Pigs were observed and rectal temperatures were recorded daily for three weeks. Vaccine efficacy was evaluated by challenging pigs with PRV after intranasal or intramuscular vaccination with SV/PRV. Weaned pigs were vaccinated one time intramuscularly with a standard 2 ml field dose of SV/PRV, PR-Vac®[b], or PRV/Marker®[a], and then challenged with a virulent strain of PRV (VDL 4892) 4 weeks post-vaccination[4]. Three day old pigs were vaccinated intranasally with a minimal protective dose of SV/PRV and then challenged with the VDL 4892 strain of PRV three weeks post-vaccination. All challenged pigs were observed daily for clinical signs of pseudorabies. No abnormalities were observed post-vaccination in any of the pigs. On average, the gilts farrowed normal sized litters and no mummified fetuses were observed. The intracerebrally inoculated pigs appeared normal and maintained normal rectal temperatures throughout the trial. The SV/PRV vaccinated and challenged pigs remained clinically healthy after challenge.

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[a] SyntroVet, Incorporated, Lenexa, Kansas.
[b] SmithKline Beecham, Lincoln, Nebraska.

INTRODUCTION

Pseudorabies (PR) is an economically important disease for the swine industry.[32] The average cost of PR for a farrow-to-finish producer has been estimated at $22.66/sow/year to $74.75/sow/year.[10] Depending on the age, reproductive status, and immune status of the pigs when they are infected with the pseudorabies virus (PRV), pigs may appear clinically normal or have a range of clinical signs from mild respiratory signs to death.[22] In the breeding age pig, the greatest economic loss is due to reproductive failure. Depending on the stage of gestation, pregnant swine may resorb their fetuses, abort, or deliver mummified, dead, weak, or healthy pigs.[13,22,38]

Virulence of PRV has been associated with several portions of the genome, and include the inverted repeat region, and the thymidine kinase (TK), and glycoprotein I (gpI) genes.[1,3,15,17,24] Thymidine kinase plays an important role in the virulence of PRV as it is partially responsible for the virus' ability to replicate in neural tissue. Incoordination, seizures, and paralysis are associated with viral replication in nervous tissue. Inoculation of pigs with PRV intracerebrally results in a rapidly fatal illness.[5] When the TK gene is removed from the PRV genome, the virulence of the virus is decreased.[17] This same phenomenon is seen when gpI[1], or portions of the inverted repeat region are removed from the viral genome.[3]

In order to reduce the severity of disease associated with PRV infections, intensive vaccination programs have been developed and implemented in herds with PR.[8,19,21,30] Several vaccines have been produced with deletions of the viral genome so that the virulence of the vaccine virus is reduced and

the vaccines are safe to use in swine. Additional diagnostic deletions have been made in the genome so that PRV vaccinated pigs can be distinguished from field infected pigs using serological enzyme-linked immunsorbent assays (ELISA).[20,33,35,36] Nursing pigs are protected against PRV infections via colostral antibodies when the pigs are nursing field infected or vaccinated dams.[6,18,23,25,34] This passive immunity has made it difficult to determine the best time to vaccinate young pigs in order to avoid interference in the development of active immunity.[5] Intranasal vaccination has been used in some herds to increase the amount of protective antibody at the site of initial virus entry and replication, and to enhance the active immune response when passive immunity is present.[7,34]

SV/PRV is an experimental modified live virus (MLV) vaccine that has been developed for use in swine. Several deletions have been made in the vaccine virus genome in order to reduce the virulence of the virus and to allow differentiation of SV/PRV vaccinated pigs from field infected pigs. Deletions of the TK gene and a portion of the internal inverted repeat were made to reduce the virulence. Diagnostic deletions were made by removing the gpI and gpX genes. Animals vaccinated with SV/PRV will not produce gpI or gpX antibodies, and consequently, will be seronegative on the HerdChek® Anti-PRV gpX[c] and HerdChek® Anti-PRV gI[c] assays. The purpose of this research was to evaluate the safety of SV/PRV in gestating gilts and young pigs, and to evaluate the efficacy of intranasal and intramuscular vaccination.

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[c] IDEXX, Incorporated, Portland, Maine.

MATERIALS AND METHODS

General

Animals and housing

All pigs were obtained from pseudorabies free herds and were housed in secured facilities. Pigs of weanling age and older were bled at the time they were placed in isolation facilities, while the sows of nursing piglets were bled at the time the piglets were started on an experiment. Pigs were bled from the eye via the orbital sinus or via the cranial vena cava.

Virus

The virus strain used in the challenge experiments was the pneumotropic strain VDL 4892. This virus was grown on Madin-Darby bovine kidney (MDBK) cells, harvested, and stored at -70°C until needed. The challenge virus was titered each time pigs were challenged. This was accomplished by making ten fold dilutions of the challenge virus. Eight wells of each dilution containing 50 µl of virus and 150 µl of MDBK cells were incubated at 37°C for 48 hours and then examined for typical cytopathic changes. The titer was determined using the Kärber method.[26] The virus was given intranasally using a 3 cc syringe which was fitted with a 16 gauge needle that had been shortened to approximately 5 mm in length. Rubber tubing[d] of size 1.6 mm X 0.8 mm was cut into a 35 mm piece and placed over the cut needle.

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[d] Fischer Scientific Company, Eden Prairie, Minnesota.

Vaccines

PRV/Marker and the experimental vaccine SV/PRV are both MLV vaccines and were supplied by SyntroVet, Inc. SmithKline Beecham's gpI deleted vaccine PR-Vac is a MLV vaccine and was purchased through a local vaccine supplier.

Serology

Serum samples were tested with commercially available PRV ELISA kits. These kits included the HerdChek® Anti-PRV Screen test[e], which is designed for use in nonvaccinated pigs, the HerdChek Anti-PRV-gpX test for animals vaccinated with PRV/Marker or SV/PRV, the HerdChek Anti-PRV-gI test for animals vaccinated with SV/PRV and PR-Vac, and the the ClinEase-PRV test[f] for animals vaccinated with the gI deleted vaccines PR-Vac or SV/PRV. All day zero serum samples were tested on the screening ELISA test to establish that the pigs were seronegative for PRV antibodies. Antibody titers were determined using a slightly modified version of the serum-virus neutralization (SVN) test that is described by Hill et al.[12]

Nasal swabs

Sterile dacron swabs[g] were moistened in Earles[h] media supplemented with sodium bicarbonate and antibiotics (1000 µg/ml of amphotericin B[i] and 50 µg/ml of gentamicin sulfate[j]). Both nostrils were swabbed with a single swab.

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[e] IDEXX Incorporated, Portland, Maine.
[f] SmithKline Beecham, Lincoln, Nebraska.
[g] Baxter Scientific, Minneapolis, Minnesota.
[h] Grand Island Biologics Company, Grand Island, New York.
[i] Squibb and Sons, Incorporated, Rolling Meadow, Illinois.
[j] Schering Veterinary Corporation, Omaha, Nebraska.

The swab was then placed into a tube containing 2 ml of the supplemented Earles.

Virus isolation

Tissue homogenates and nasal swab media were centrifuged at 2000 x g for 15 minutes. The supernatant was transferred to clean tubes and 200 µl was placed in duplicate wells of 24 well cell culture plates containing MDBK cells. Tubes were then stored at -70°C. Samples were incubated for 1-1.5 hours at 37°C. The media was removed from each well and replaced with Eagle's minimum essential media (MEM)[h] containing 2% fetal calf serum, 1000 µg/ml of amphotericin B, and 50 µg/ml of gentamicin sulfate. Plates were observed daily for one week for typical cytopathic cell changes. All plates containing wells with no viral activity were frozen at -70°C and then thawed at 37°C. Media from duplicate wells were mixed together and 200 µl of this mixed media was inoculated in duplicate on 24 well cell culture plates. Wells were observed for an additional week and then discarded.

Virus detection in tissues of dead pigs

All challenged pigs that died were necropsied and tissues were collected for detection of PRV. Tonsils were collected for the fluorescent antibody test[12], while brain, lung, and spleen were collected for virus isolation.

Plaque assay

The plaque assay technique used to determine virus excretion via the nose is a slightly modified version of the technique described by Dulbecco.[9] All sample dilutions were inoculated into three different 60 mm cell culture

dishes. Only dishes with ≥10 plaques and ≤100 plaques were used for titration calculations. The plaque titer was determined by dividing the the average plaque count by the inoculum volume (0.1 ml) and by the dilution factor.

Trial 1: Safety in gestating gilts

Eleven gilts which were 40 to 75 days into gestation were inoculated intravenously with a single 2 ml field dose of SV/PRV. The gilts were bled at the time of inoculation and 4 weeks post-vaccination. Gilts were observed daily from the time of inoculation until the time of farrowing.

Trial 2: Safety in 3 day old pigs

Four, three day old pigs were inoculated intracerebrally with a field dose of SV/PRV that was reconstituted to 0.2 ml/dose. The remainder of the pigs in the litter were kept as contact controls. All pigs were weighed on days 0, 7, and 21. The pigs were observed and rectal temperatures were recorded daily for 21 days. All pigs in the litter were bled at the end of the study.

Trial 3: Efficacy of a minimal dose intranasal vaccine

Twenty pigs (no. 376-395) were given a single 2 ml minimal dose (1/100th of the standard field dose) of SV/PRV intranasally at three days of age. Each pig received 1 ml/nostril. Nasal swabs were collected on days 3, 6, 9, and 12 post-vaccination and processed for virus isolation. Twenty-three days post-vaccination, the twenty vaccinated and five nonvaccinated control pigs (no. 396-400) were challenged intranasally with a 1 ml dose ($2.3X10^5$ $TCID_{50}$/ml) of VDL 4892 PRV. Each pig received 0.5 ml/nostril. Nasal swabs were collected on days 3, 6, 9, and 12 post-challenge and virus excretion was quantitated using

a plaque assay method. Pigs were bled at the time of challenge and on days 4, 7, 10, 14, and 23 post-challenge. Pigs were observed daily and clinical signs were recorded.

Trial 4: Efficacy of a standard dose intramuscular vaccine

Four groups of 10 weaned pigs were vaccinated intramuscularly with either PRV/Marker (no. 51-60), SV/PRV (no. 61-70), PR-Vac (no. 71-80) according to the manufacturer's directions, or were kept as nonvaccinated controls (no. 81-90). Each group was housed separately. The vaccinated pigs were bled 21 days post-vaccination and prior to challenge. All pigs were challenged with a 1 ml dose ($2.3X10^5$ $TCID_{50}$/ml) of VDL 4892 PRV on day 31. Each pig received 0.5 ml/nostril. Pigs were observed daily and nasal swabs were collected on days 3, 6, 9, and 12 post-challenge. Virus excretion was quantitated using a plaque assay method. All pigs were bled on days 7, 14, and 20 post-challenge.

RESULTS

Trial 1: Safety in gestating gilts

All eleven gilts remained clinically normal throughout gestation, and no adverse reactions were observed after the intravenous inoculation. The gilts had what would be considered normal litters (Table 1). The eleven gilts averaged 9.1 pigs born and 8.0 pigs born live. All eleven gilts were seropositive for pseudorabies antibodies on the screening ELISA test four weeks post-vaccination, but were seronegative for gpI on the HerdChek gI differential test. The serum-virus neutralization antibody titers were 1:4 or less.

Table 1. Farrowing data for vaccinated gilts

Gilt #	Inoculated	Farrowed	Born Live	Born Dead
57	11/16/90	12/26/90	11	0
126	11/16/90	12/31/90	5	0
128	11/16/90	1/5/91	9	0
129	11/16/90	1/2/91	9	1
130	11/16/90	1/18/91	9	1
131	11/16/90	1/19/91	8	0
133	11/16/90	1/23/91	8	5
156	11/16/90	1/23/91	5	1
158	11/16/90	1/24/91	9	0
161	11/16/90	12/5/90	7	1
168	11/16/90	1/27/91	9	2

Trial 2: Safety in 3 day old pigs

The four vaccinated pigs and four contact control littermates remained clinically normal throughout the three week observation period. The range of rectal temperatures for the vaccinated pigs was 101.5-104.5 °F, while the range for the control pigs was 101.0-104.2 °F. The vaccinated pigs had average rectal temperatures (°F) of 103.0, 102.7, 102.7, and 102.8, and the control pigs had average rectal temperatures of 102.7, 102.2, 102.3, and 102.2.

Both groups of pigs gained weight throughout the trial. The smallest gain was seen during the first seven days of the trial, and then the pigs rapidly gained weight until the end of the trial (Table 2). Blood was collected from the pigs on day 21 of the trial. Two of the vaccinated pigs showed a weak antibody response on the screening and latex agglutination tests, but were seronegative on the three differential tests and the SVN test.

Table 2. Weight gains of intracerebrally vaccinated and control pigs

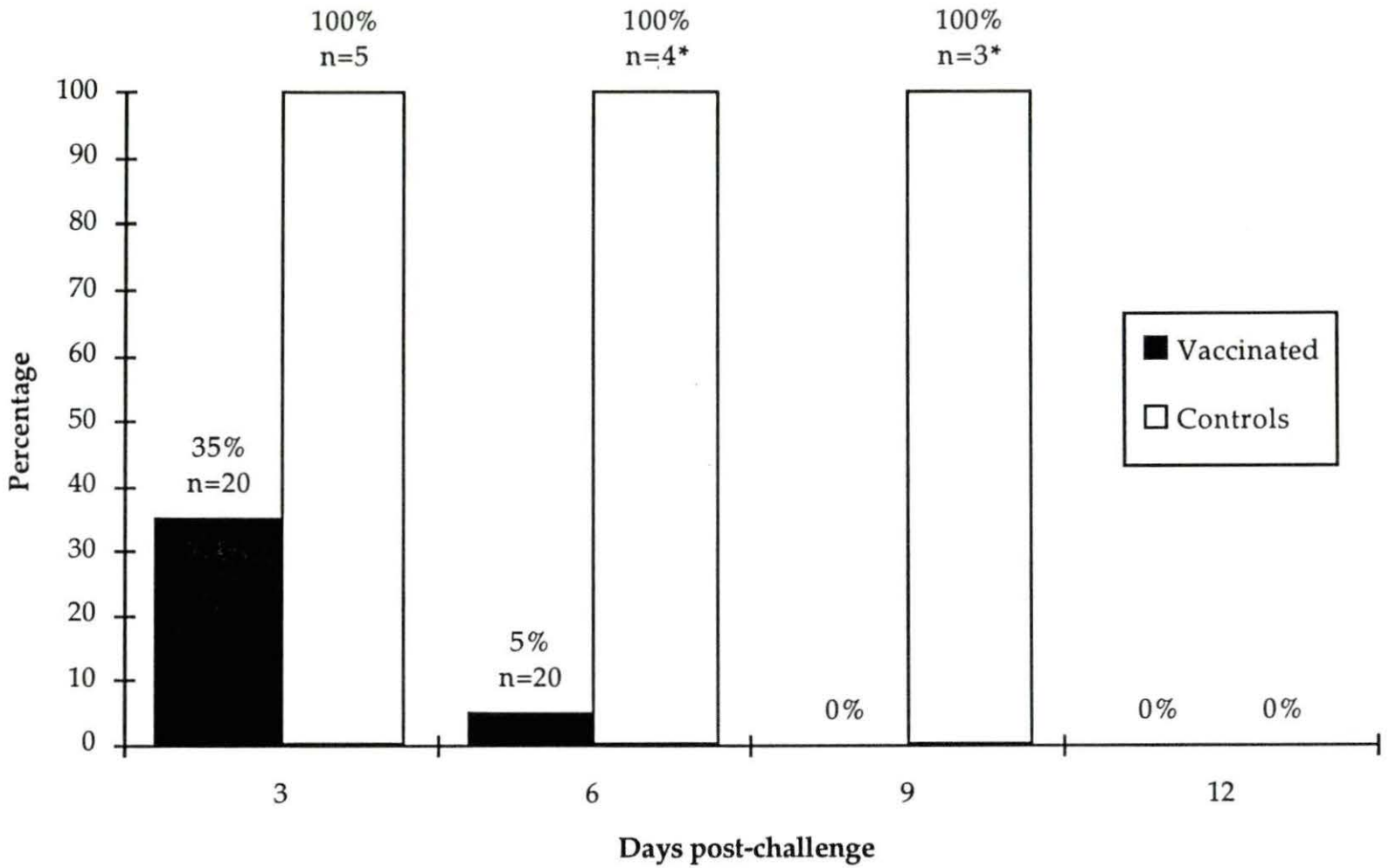
Parameter Evaluated	Vaccinated	Controls
Weight gain (range), 0-7 days (lbs)	1.5-2.5	0.5-2.5
Average weight gain (lbs/day)	0.28	0.22
Weight gain (range), 7-14 days (lbs)	2.5-4.0	3.0-4.5
Average weight gain (lbs/day)	0.48	0.53
Weight gain (range), 14-21 days (lbs)	3.5-4.5	3.0-5.0
Average weight gain (lbs/day)	0.61	0.51
21 day average weight gain (lbs/day)	0.45	0.43

Trial 3: Efficacy of a minimal dose intranasal vaccine

Of the twenty vaccinated pigs, three shed virus during the first six days post-vaccination. The vaccinated pigs appeared clinically normal post-vaccination and post-challenge. Two nonvaccinated pigs developed a nasal discharge 3 days post-challenge and then proceeded to have central nervous system (CNS) signs of incoordination and seizures. One of these pigs died on day 6 post-challenge and the other pig died on day 8 post-challenge. The tonsils of both pigs were positive for PRV on the fluorescent antibody test. Virus was isolated from the brains of both pigs, but not from the lungs or spleens. The remaining three control pigs began having clinical signs of PR on day 5 post-challenge. The three remaining control pigs all had depression, dyspnea, nasal discharge, incoordination, tremors, and seizures. Two of the three remaining control pigs continued to have tremors and were incoordinated throughout the 14 day observation period.

Quantitation of virus excreted from vaccinated and nonvaccinated pigs post-challenge revealed that vaccinated pigs excreted less virus, with an average of 10^1 PFU/ml of virus on day 3 post-challenge, while the nonvaccinated pigs excreted $10^{2.5}$ PFU/ml of virus. An analysis of variance[27] revealed that the amount of virus shed was significantly reduced ($p<0.03$) in the vaccinated pigs compared to the control pigs. In addition to shedding less virus, the vaccinated pigs shed virus for a shorter length of time (Figure 1).

All sera collected were tested for the presence of PRV antibodies using the SVN, gpX, and both gI tests. The vaccinated and control pigs were seronegative on the differential tests until day 10 post-challenge, with the exception of control pig 397, which tested as a suspect on the HerdChek gI test



*Smaller number (n) due to death of pigs.

Figure 1. Intranasal vaccination - percentage of pigs shedding virus post-challenge

on day 7 post-challenge. The remainder of the pigs tested positive for PRV antibodies on the differential tests beginning on day 10 post-challenge (Table 3; Figures 2, 3, and 4). The vaccinated pigs had SVN titers of 1:8 or less through day 4 post-challenge, and the control pigs were seronegative through day 10 post-challenge. By day 7 post-challenge, the vaccinated pigs had rising antibody titers (Table 4).

Trial 4: Efficacy of a standard dose intramuscular vaccine

The vaccinated pigs appeared clinically normal post-vaccination and post-challenge with the exception of SV/PRV vaccinated pig 66 and PR-Vac vaccinated pig 77. Pig 66 developed swollen joints and appeared to be lame on day 9 post-challenge. A draining lesion developed on the left, lateral thigh and the pig had difficulty rising. This animal was euthanized on day 11 post-challenge. Pig 77 developed tremors and was incoordinated on day 5 post-challenge. This pig continued to have tremors for an additional two days before it returned to normal.

The nonvaccinated pigs began showing clinical signs of nasal discharge and sneezing by day 3 post-challenge (Figure 5). Pig 81 died acutely on day 6 post-challenge after having appeared to be normal except for excessive sneezing. One pig developed a minor case of incoordination which was present only on day 6 post-challenge. Another pig developed a purulent nasal discharge and had difficultly breathing, but did not develop any CNS signs. Pig 90 appeared clinically normal throughout the 14 day observation period. By day 6 post-challenge, the remainder of the pigs developed CNS signs which included incoordination, head tilt, tremors, and seizures. Pigs 84, 82, 85, and 89 died on

Table 3. Intranasal vaccination - differential serology test results

Group	Days Post-Challenge		HerdChek gpX	HerdChek gI	ClinEase gI
Vaccinated	10	P[1]	0/20	2/20	13/20
		S[2]	0/20	10/20	2/20
			0	12	15
	14	P	2/20	9/20	18/20
		S	2/20	6/20	1/20
			4	15	19
	23	P	12/20	16/20	19/20
		S	3/20	2/20	1/20
			15	18	20
Controls	10	P	3/3	2/3	3/3
		S		1/3	
	14	P	3/3	1/3	3/3
		S		2/3	
	23	P	3/3	3/3	3/3
		S			

[1]P=number of positive samples /total number of samples tested.
[2]S=number of suspect samples/total number of samples tested.

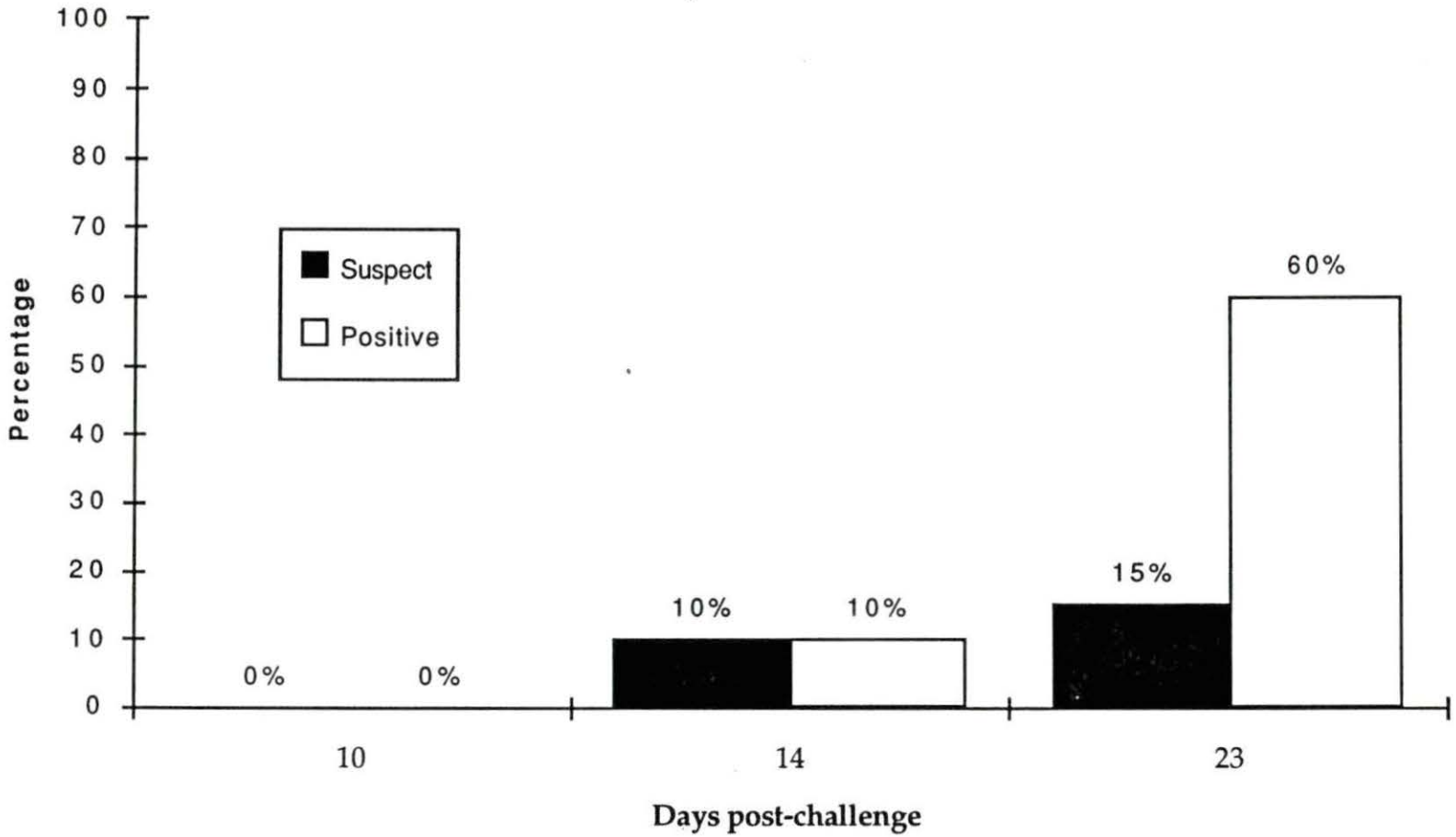


Figure 2. Intranasal vaccination followed by challenge - HerdChek gpX serology (n=20)

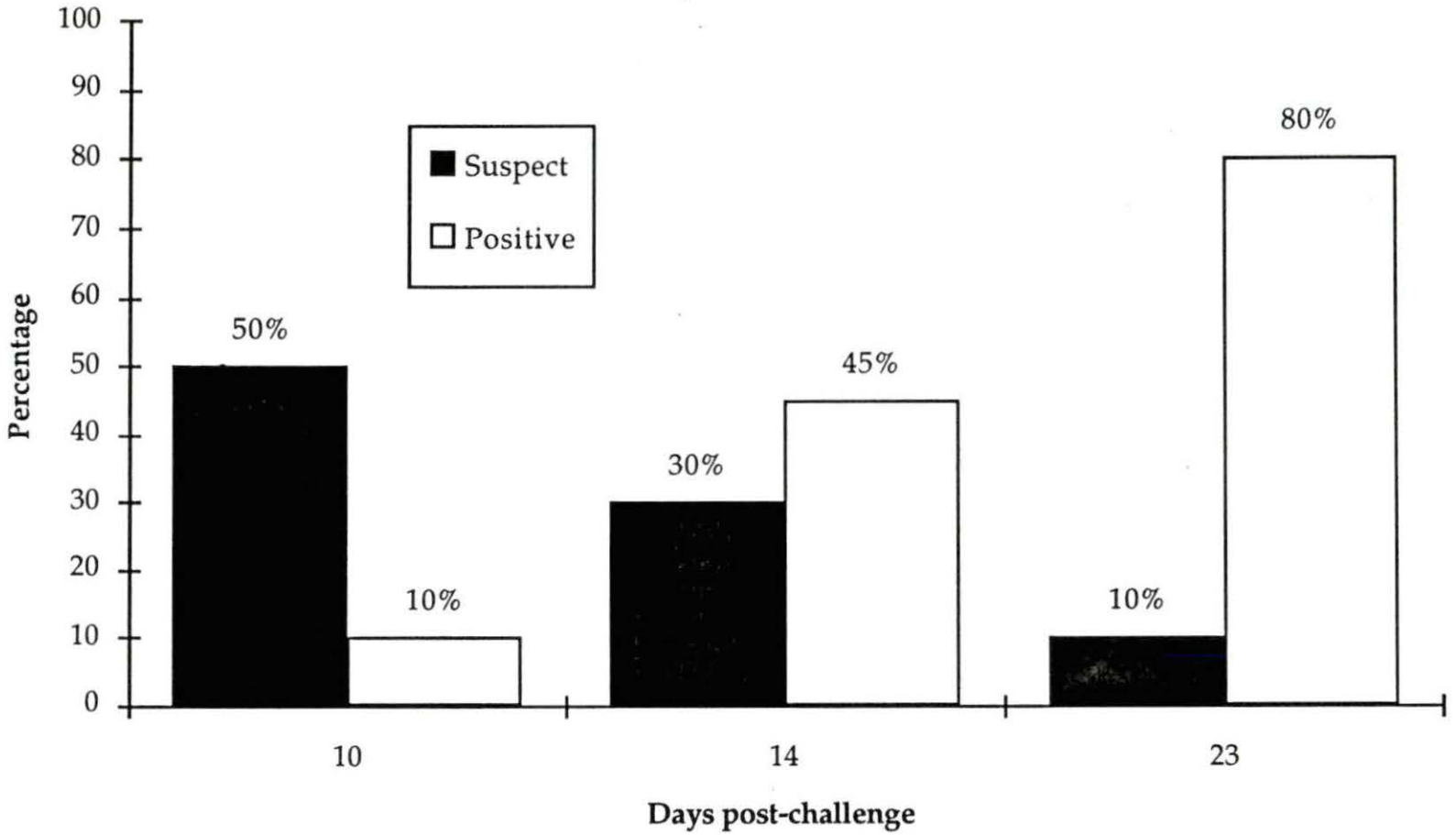


Figure 3. Intranasal vaccination followed by challenge - HerdChek gI serology (n=20)

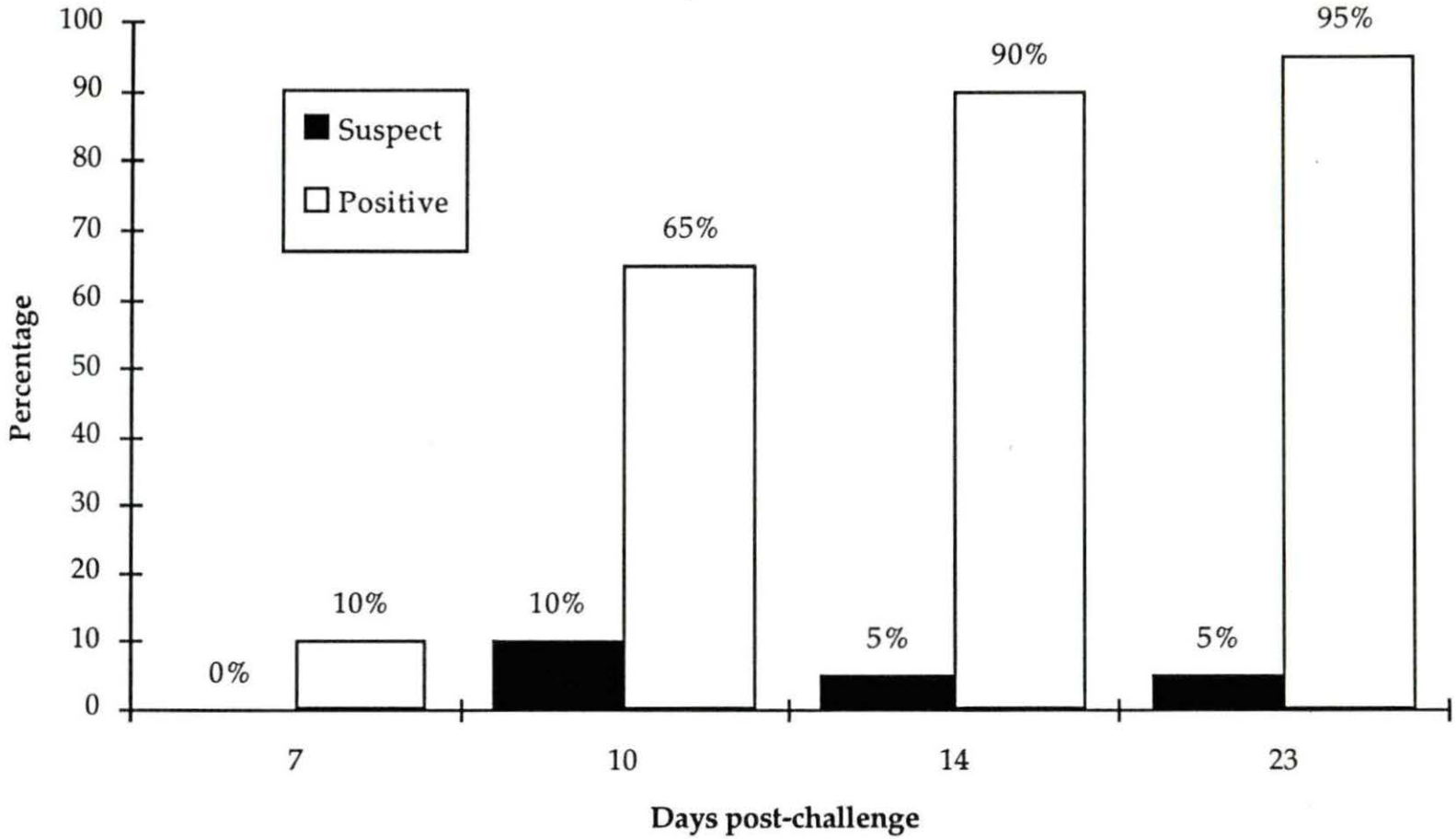


Figure 4. Intranasal vaccination followed by challenge - ClinEase gI serology (n=20)

Table 4. Intranasal vaccination - serum-virus neutralization antibody titers

Group	Day 23 (CH)[1]	Day 27 (4PC)[2]	Day 30 (7PC)	Day 33 (10PC)	Day 37 (14PC)	Day 46 (23PC)
Vaccinated						
376	<2	<2	16	64	256	64
377	2	2	1024	128	256	64
378	<2	<2	32	128	64	32
379	<2	4	64	128	128	64
380	2	<2	64	128	256	128
381	<2	2	16	32	32	16
382	<2	8	8	16	16	8
383	4	2	4	4	16	8
384	<2	<2	128	256	32	32
385	<2	4	2	8	8	8
386	4	2	4	4	32	8
387	<2	2	8	32	16	16
388	<2	2	32	16	32	8
389	2	2	256	512	256	128
390	<2	<2	8	32	64	32
391	2	2	128	128	128	32
392	2	4	32	32	16	32
393	<2	<2	32	64	128	32
394	<2	<2	4	4	16	8
395	<2	<2	256	128	128	128
Controls						
396	<2	<2	<2	<2	2	4
397	<2	<2	<2	<2	2	16
398	<2	<2	DEAD	DEAD	DEAD	DEAD
399	<2	<2	<2	DEAD	DEAD	DEAD
400	<2	<2	<2	<2	2	4

[1]CH=day of challenge.
[2]PC=days post-challenge.

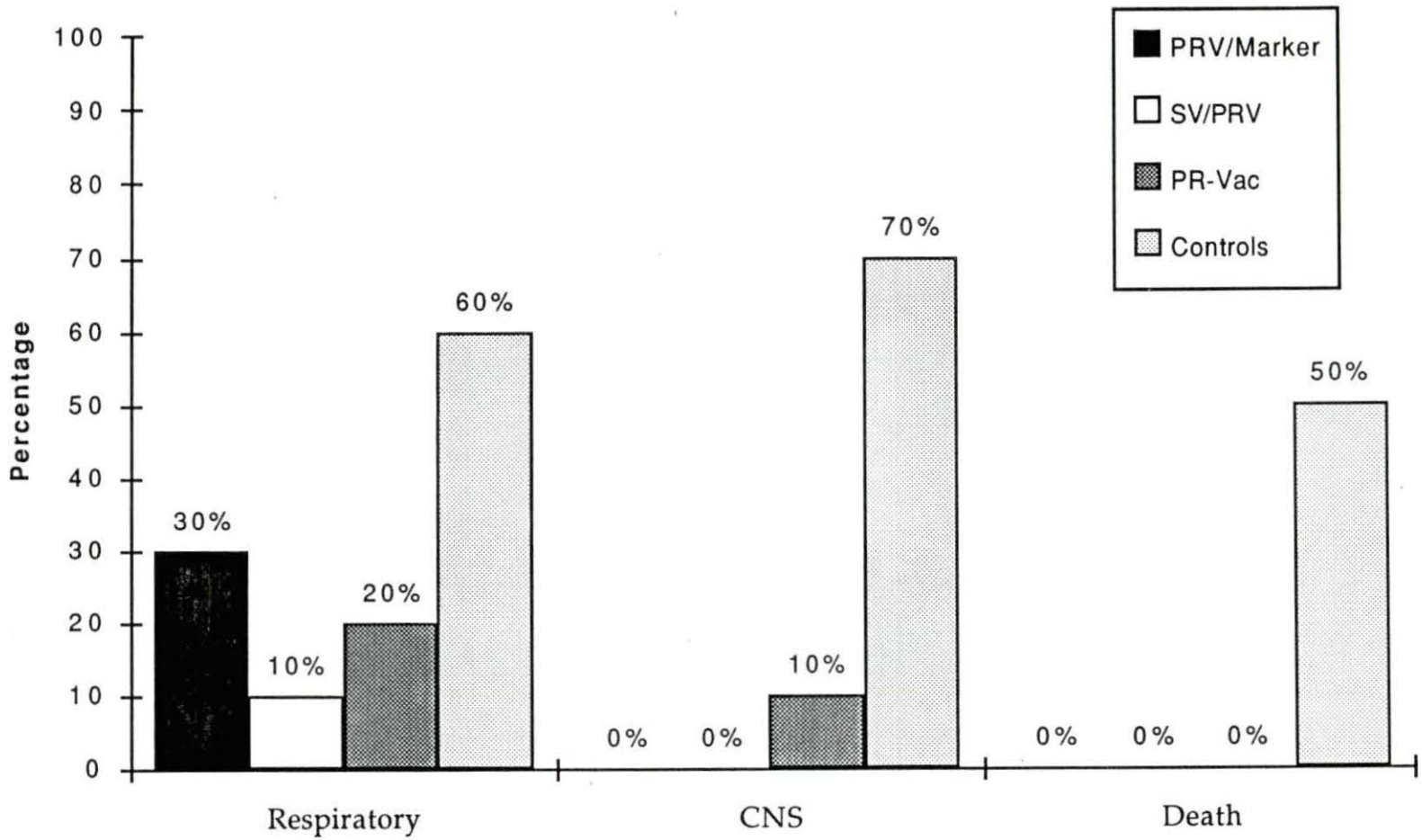


Figure 5. Intramuscular vaccination - clinical signs post-challenge (n=10 per treatment group)

days 7, 8, 9, and 9 respectively. The nonvaccinated pigs that survived did not have any CNS signs by the end of the 14 day observation period.

Virus quantitation from the nasal swabs revealed that the vaccinated pigs shed smaller quantities of virus compared to the control pigs. An analysis of variance using the Duncan multiple range test[28] revealed a significant difference ($p<0.03$) in the amount of virus shed. The average amount of virus shed on day 3 post-challenge by the vaccinated pigs was $10^{1.5}$ PFU/ml for the PRV/Marker pigs and $10^{1.7}$ PFU/ml for both the PR-Vac and SV/PRV pigs. In contrast, the average titer of virus shed by the control pigs on day 3 post-challenge was $10^{2.5}$ PFU/ml. The vaccinated and control pigs were no longer shedding virus by day 9 post-challenge (Figure 6).

The PRV/Marker and SV/PRV vaccinated pig sera were tested for the presence of gpX antibody and the PR-Vac and SV/PRV sera were tested for the presence of gI antibody (Tables 5 & 6; Figures 7, 8, and 9). Two days before challenge, SV/PRV pig 62 tested positive for gI antibodies on both gI tests and negative on the gpX test. These tests were repeated several times on the serum sample, with the same results. Serum collected from this pig 10 days before challenge and 7 days post-challenge was negative for gpX and gI antibodies. All sera collected were tested for the presence of PRV antibodies using the SVN test (Table 7). Two days prior to challenge, one PRV/Marker and one SV/PRV vaccinated pig had SVN antibody titers of 1:2. The remainder of the vaccinated and all of the control pigs had SVN antibody titers of <2. The control pigs did not develop SVN antibody titers until day 14 post-challenge. The SVN antibody titers for the control pigs ranged from 1:4 to 1:16.

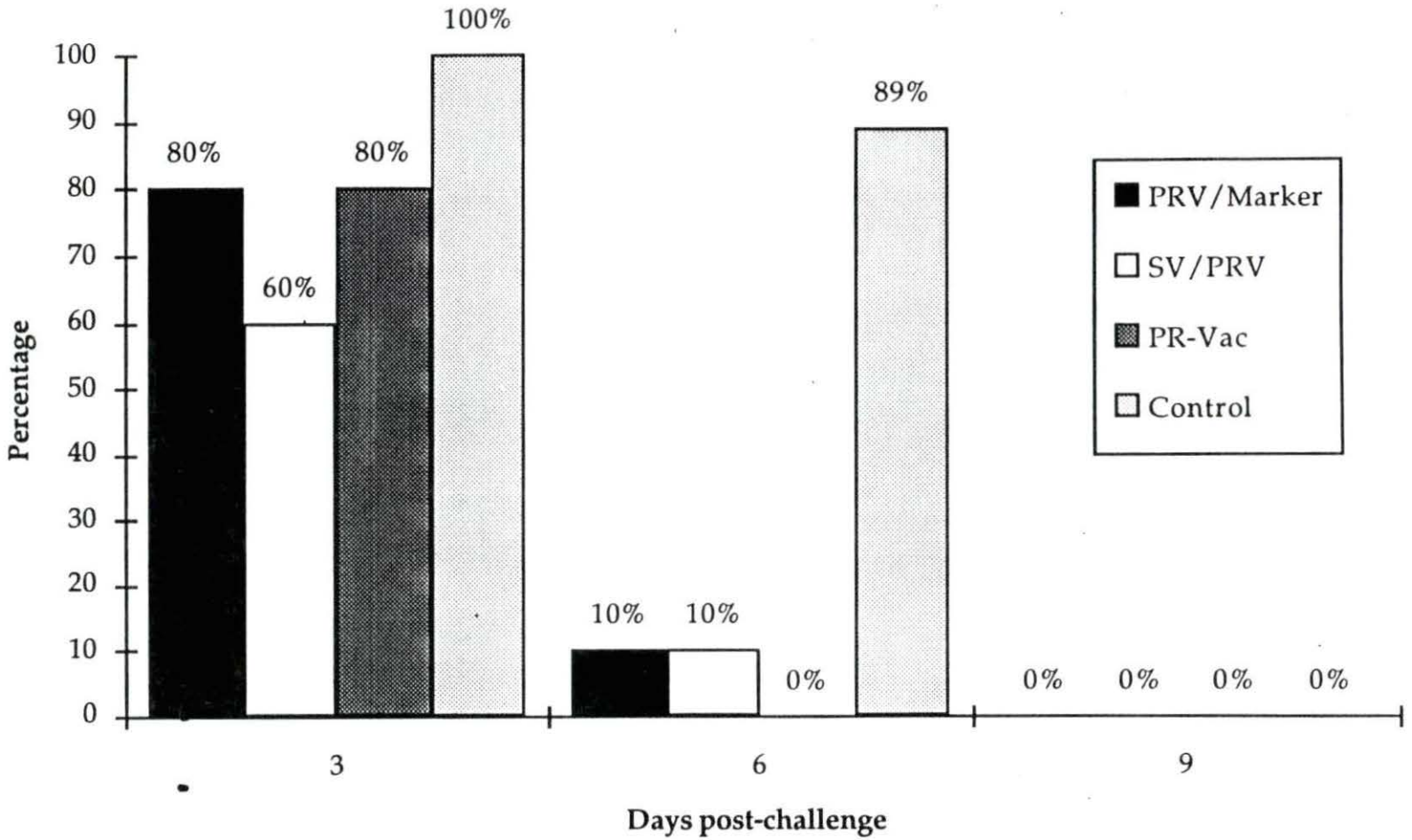


Figure 6. Intramuscular vaccination - percentage of pigs shedding virus post-challenge (n= 10 per treatment group)

Table 5. Intramuscular vaccination - differential serology test results

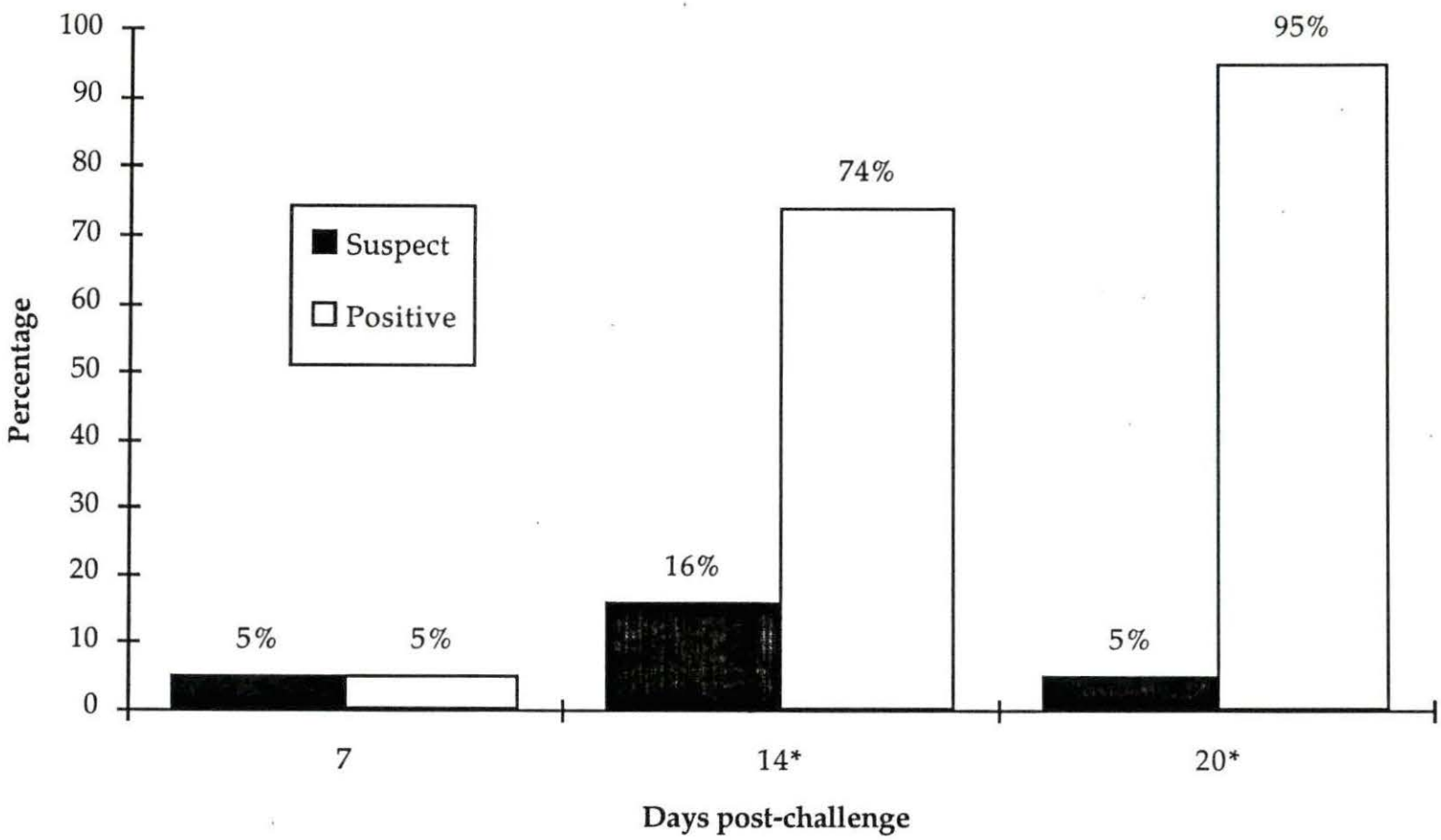
Group	Days Post-Challenge		HerdChek gpX	HerdChek gI	ClinEase gI
PRV/Marker	7	P[1]	1/10		
		S[2]	1/10		
			2		
	14	P	7/10		
		S	2/10		
			9		
	20	P	10/10		
		S	0/10		
			10		
SV/PRV	7	P	0/10	0/10	2/10
		S	0/10	0/10	2/10
			0	0	4
	14[3]	P	7/9	8/9	9/9
		S	1/9	1/9	0/9
			8	9	9
	20	P	8/9	9/9	9/9
		S	1/9	0/9	0/9
			9	9	9
Controls	7	P	1/8	0/8	0/8
		S			
	14	P[4]	5/5	5/5	5/5
		S			
	20	P	5/5	5/5	5/5
		S			

[1]P=number of positive samples /total number of samples tested.
[2]S=number of suspect samples/total number of samples tested.
[3]Pig 66 euthanized day 11 post-challenge.
[4]Three pigs died prior to day 14 post-challenge.

Table 6. Intramuscular vaccination - differential serology test results

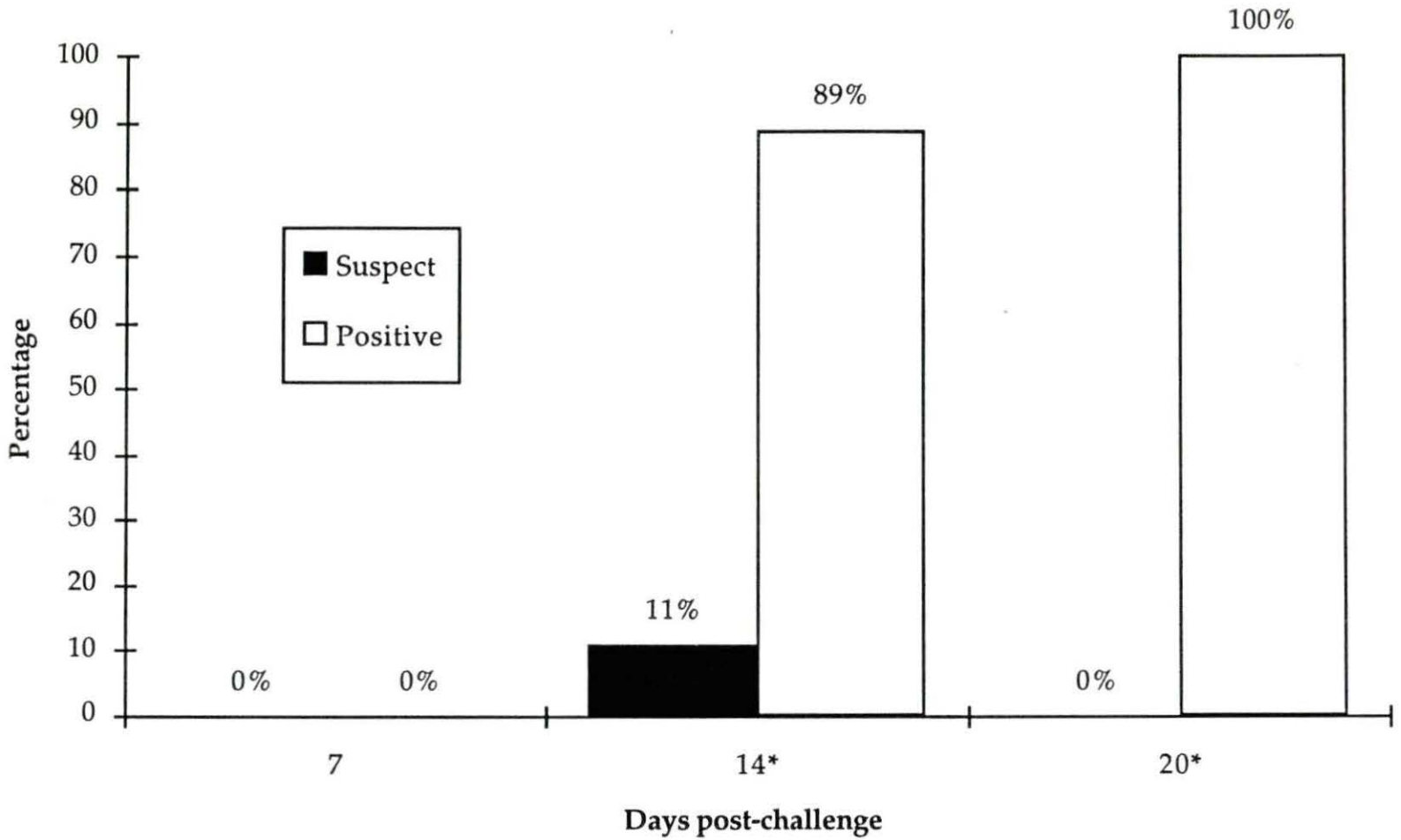
Group	Days Post-Challenge		HerdChek gpX	HerdChek gI	ClinEase gI
PR-Vac	7	P[1]		0/10	3/10
		S[2]		0/10	1/10
				0	4
	14	P		9/10	10/10
		S		1/10	0/10
				10	10
	20	P		10/10	10/10
		S		0/10	0/10
				10	10
SV/PRV	7	P	0/10	0/10	2/10
		S	0/10	0/10	2/10
			0	0	4
	14[3]	P	7/9	8/9	9/9
		S	1/9	1/9	0/9
			8	9	9
	20	P	8/9	9/9	9/9
		S	1/9	0/9	0/9
			9	9	9
Controls	7	P	1/8	0/8	0/8
		S			
	14	P[4]	5/5	5/5	5/5
		S			
	20	P	5/5	5/5	5/5
		S			

[1]P=number of positive samples /total number of samples tested.
[2]S=number of suspect samples/total number of samples tested.
[3]Pig 66 euthanized day 11 post-challenge.
[4]Three pigs died prior to day 14 post-challenge.



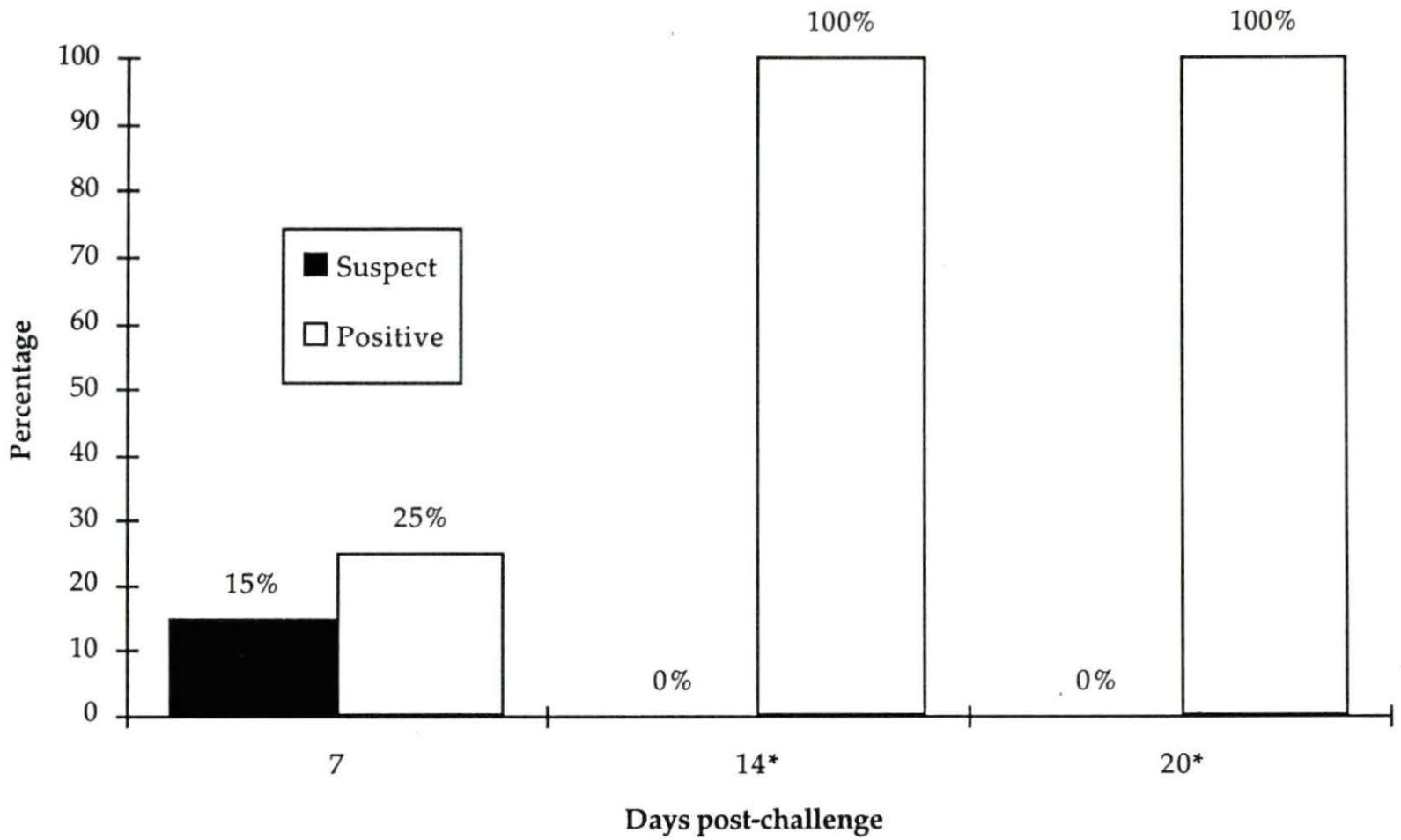
*n=19 for days 14 and 20 post-challenge due to euthanasia of pig 66 on day 11.

Figure 7. Intramuscular vaccination followed by challenge - HerdChek gpX serology (n=20)



*n=19 for days 14 and 20 post-challenge due to euthanasia of pig 66 on day 11.

Figure 8. Intramuscular vaccination followed by challenge - HerdChek gI serology (n=20)



*n=19 for days 14 and 20 post-challenge due to euthanasia of pig 66 on day 11.

Figure 9. Intramuscular vaccination followed by challenge - ClinEase gI serology (n=20)

Table 7. Intramuscular vaccination - serum-virus neutralization antibody titers

Group	Day 38 (7PC)[1]	Day 45 (14PC)	Day 51 (20PC)
PRV/Marker	128	256	256
	64	512	256
	256	256	256
	64	128	128
	512	512	256
	32	128	128
	256	256	256
	512	1024	1024
	128	64	128
	256	256	256
Mean	221	339	294
SV/PRV	512	512	512
	128	256	256
	512	512	256
	128	256	256
	2048	1024	1024
	256	DEAD	DEAD
	256	256	256
	512	512	256
	32	512	512
	256	256	256
Mean	464	455	398
PR-Vac	2048	2048	1024
	256	256	256
	512	256	256
	1024	128	512
	2048	512	1024
	1024	512	512
	512	256	256
	256	512	256
	512	1024	512
	512	512	512
Mean	870	602	512

[1]PC=post-challenge

DISCUSSION

Trial 1: Safety in gestating gilts

The average number of pigs born to the vaccinated gilts was 9.1, while the average number of pigs born during the same time period to nonvaccinated gilts and sows in the same herd was 10.2. This finding is not unexpected, as gilts generally have a lower number of pigs born compared to sows. The number of dead pigs born was comparable to the average for the herd during the same time period. Gilt 133 had more dead pigs compared to the other 10 gilts. This gilt delivered thirteen pigs, of which five were dead. The five dead pigs were fully developed and three of them were still within the fetal membranes. This trial was conducted in a field situation and the births were unattended. There is a good possibility that several of these dead pigs may have been born alive. Considering that gilt 133 had the largest litter of all 11 gilts, it is not unreasonable to assume that she became fatigued and was unable to remove the fetal membranes. It is also important to note that all of the dead pigs born to the 11 gilts were fully developed and had not undergone autolysis, indicating that the pigs died during farrowing.

Additional diagnostic work would have been helpful in confirming the safety of the vaccine in gestating sows and gilts. Serum samples collected from the pigs prior to nursing and evaluated for the presence of PRV antibody would have helped to determine if the vaccine virus was able to enter the fetal pig during gestation. In addition, tissues collected from the dead pigs could have been evaluated for the presence of virus using virus isolation and

fluorescent antibody techniques. Based on the data collected, this vaccine appears to be safe for use in gestating sows and gilts.

Trial 2: Safety in 3 day old pigs

The intracerebrally inoculated pigs and contact control pigs were clinically indistinguishable throughout the trial. Both groups of pigs gained weight at approximately the same rate. The only differences noted between the two groups was the difference in average rectal temperatures and the seroconversion by two of the vaccinated pigs on the screen test. With the exception of one pig in the control group, the average rectal temperatures were 102.3°F and lower. In contrast, the average rectal temperatures for the vaccinated pigs were 102.7°F and higher. This difference in temperatures could be due to several factors, including individual pig variation. This would account for the one control pig having an average rectal temperature of 102.7. In addition, the elevated temperatures could be due to a local reaction at the site of injection. This would account for the four vaccinated animals, but not for the one control pig.

The serology results indicate that the vaccine virus was able to undergo replication and stimulate an immune response. Tenser et al.[29] examined PRV replication in trigeminal ganglia of mice and Lomniczi et al.[16] examined PRV replication in chicken brains. Both groups showed that TK negative PRV has the ability to undergo limited replication in neural tissue. This limited replication may stimulate the immune system such that an antibody response could be detected using the screen and latex agglutination tests. In addition, vaccine virus may have been present on the needle as it was inserted into or

withdrawn from the pig. The virus could have become lodged in the skin or blood vessel as the needle was moved through these tissues. If enough virus was deposited in these areas, the virus could have replicated to a high enough level to cause the pigs to seroconvert on the screen and latex agglutination tests. Van Oirschot et al. have suggested that since pigs less than one week old are highly susceptible to PRV, the absence of clinical signs post-vaccination is a good indicator of avirulence of a vaccine.[37] If this is assumed to be true, then the clinical appearance of the pigs throughout the trial indicates that intracerebral inoculation was not detrimental to the pigs, and that the vaccine is safe for use in three day old pigs.

Trial 3: Efficacy of a minimal dose intranasal vaccine

Virus isolation attempts from post-vaccination nasal swabs showed that three of twenty intranasally vaccinated pigs were shedding virus in the nasal secretions for up to 6 days after vaccination. The virus was not quantitated to determine the level of viral secretion, but this could potentially be a problem if the vaccinated pigs are shedding enough virus to infect pigs that are in contact with the vaccinated pigs. This may result in a pig being identified as field infected if a differential test is not used when, in fact, the pig has seroconverted to vaccine virus rather than field virus.

The vaccinated pigs were protected when challenged with PRV compared to the nonvaccinated pigs. This was confirmed both clinically in the appearance of the pigs, and in the antibody titers that developed post-challenge. The protection afforded by the vaccine was significant, as the amount of virus shed and the length of time of viral shedding were both reduced. The analysis of

variance revealed that the amount of virus shed was significantly reduced in the vaccinated pigs compared to the control pigs on day 3 post-challenge. In contrast, the nonvaccinated pigs developed clinical signs of PR, had lower antibody titers, and secreted larger amounts of virus and for a longer period of time. The protection provided by SV/PRV is not only due to systemic antibodies, but is also due to the local immunoglobulin A (IgA) response that occurs at the level of the nasal mucosa. Vaccination at this site of viral entry allows for the development of high levels of IgA. This local antibody has the ability to bind to PRV and reduce the amount of viral replication, thereby reducing the severity of clinical signs due to virus dissemination throughout the body.

The serology results for the vaccinated animals indicated that both gI tests were able to detect PRV antibodies due to challenge at an earlier time than the gpX test. These results also revealed that the gpX test was not able to detect as many positive pigs as the gI tests. By three weeks post-challenge the gpX test was only detecting 75% of the pigs as positive, while the ClinEase gI test detected 100% of the pigs as positive. Studies in other laboratories indicate that the gpX antigen is produced in large amounts and is secreted into the surrounding cell media.[2,11] This would indicate that there should be enough antigen present in the pig to produce a detectable level of antibodies to gpX on the HerdChek gpX test. The fact that fewer pigs were detected using the gpX test indicates that the gpX antigen may be produced in large quantities, but it may not be as antigenic as the gI antigen. This would contradict a study done by Lens et al. in which gpX was felt to be highly antigenic.[14] It is possible that it may take much larger amounts of this viral glycoprotein to stimulate an

immune response compared to the gI antigen. It may also be that the large amount of gpX produced results in the gpX antibodies being rapidly bound to the gpX antigen and removed from the circulation. This could result in gpX antibody levels that would be too low to detect on the gpX test. An alternative hypothesis is that the gpX test is not as sensitive as the gI tests. If SV/PRV is licensed for use in the United States, it would be licensed for use with both HerdChek tests. This could lead to a diagnostic dilemma in determining which test to use to accurately identify infected pigs and in determining the infection status in pigs which are positive on one test and negative on the other test.

Trial 4: Efficacy of a standard dose intramuscular vaccine

The vaccinated pigs clinically appeared to be protected by the respective vaccines when compared to the nonvaccinated pigs There was no difference in clinical signs between vaccine groups other than one PR-Vac pig developed some incoordination. Both the vaccinated and control pigs shed virus for the first six days post-challenge, but by day 6 there were fewer vaccinated pigs shedding virus compared to the nonvaccinated pigs. The analysis of variance revealed a significant difference in the amount of virus shed by the vaccinated animals compared to the control animals. There was no significant difference in the amount of virus shed by each group of vaccinated pigs.

The vaccinated pigs rapidly developed high antibody titers on the SVN test following challenge and the antibody titers remained high through the final bleeding three weeks post-challenge. With the exception of three samples on the gpX test, all of the pigs were seropositive on the gpX and both gI tests by 14

days post-challenge. This indicates that the intramuscular vaccines are able to prime the immune system so that there is a rapid production of antibodies following challenge. The pigs that were vaccinated intramuscularly and then challenged were identified as PRV exposed pigs on the gpX and both of the gI tests at an earlier time post-challenge compared to the intranasally vaccinated and challenged pigs. All of the intramuscularly vaccinated pigs seroconverted on the three differential tests whereas not all of the intranasally vaccinated pigs seroconverted on these tests. This may indicate that the route of exposure to vaccine virus can affect the rate of seroconversion on the tests. This may be partly due to the local immune response at the site of inoculation.

The serum samples from each bleeding were tested at the end of the trial in order to prevent altered test results due to day to day variation of the tests. Had the samples been tested each time they were collected, the sample that was identified as coming from SV/PRV vaccinated pig 62 two days prior to challenge would have been identified sooner as positive on both gI tests and negative on the gpX test. This would have allowed for a second blood sample to be collected prior to challenge to determine if the pig was truly positive for pseudorabies gI and gpX antibodies. Considering that the pig was negative on all three differential tests ten days prior to challenge and seven days post-challenge, it is felt that this pig was not infected with PRV prior to challenge. This is also confirmed by the fact that two days prior to challenge, the sample was negative on the gpX test and positive on the gI tests. This would indicate a possible misidentification of the sample. The serum that was tested probably came from a PRV/Marker vaccinated pig rather than the SV/PRV vaccinated pig.

CONCLUSIONS

Vaccination with SV/PRV intravenously, intracerebrally, intramuscularly, or intranasally did not result in any adverse effects to the pigs. In addition, vaccination of both young and old, as well as pregnant pigs, did not result in any detrimental effects to the pigs. Additional information such as assessing tissues of newborn dead pigs for vaccine virus and determining the rate of virus transmission to control pigs that were in contact with intranasally vaccinated pigs would have further enhanced the data already presented. Although the vaccine was not able to prevent infection with PRV, it did significantly reduce the amount of virus shed, the length of time that virus was shed, and it reduced the severity of clinical signs associated with infection. The intranasal route of vaccination resulted in fewer animals shedding virus on day three compared to the intramuscular route of vaccination. This has also been seen in other studies[6,8,19] and is most likely due to the local humoral immune response of Ig A. The vaccine also stimulated the production of high serum neutralizing antibody titers post-challenge. Based on the results of the four trials, the SV/PRV vaccine appears to be both safe and efficacious for use in swine of all ages and stages of gestation.

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Section II. EVALUATION OF A DOUBLE GENE DELETED PSEUDORABIES VACCINE AND ITS ASSOCIATED COMPANION DIAGNOSTIC TESTS

SUMMARY

The experimental modified live virus (MLV) pseudorabies vaccine SV/PRV contains two differential diagnostic deletions, gpI and gpX. Two additional deletions, in the repeat region and in the thymidine kinase gene, were made for the purpose of attenuation. In the first trial, pigs were vaccinated multiple times with SV/PRV to determine if multiple vaccinations would cause the animals to seroconvert on the companion diagnostic tests. The pigs were then challenged with the virulent VDL 4892 strain of pseudorabies virus.[2] Serum samples were collected post-challenge to evaluate seroconversion on the companion diagnostic tests. In the second trial, pigs vaccinated with licensed vaccines that had deletions of gpI or gpX were vaccinated with SV/PRV to determine if the animals would remain seronegative to the companion diagnostic tests. All of the pigs remained seronegative to the gpX and both gI tests. The pigs that were subsequently challenged seroconverted to the gpX and both gI tests.

INTRODUCTION

Pseudorabies (PR) can be an economically devastating disease for swine producers. The etiologic agent is the pseudorabies virus (PRV). Infections with this virus can cause losses due to deaths, poor weight gains, secondary bacterial infections, infertility, and a decrease in the number of pigs born alive.[4] To reduce the losses from PRV infections, many producers use either killed or MLV PR vaccines. These vaccines have helped to reduce the severity of the disease and reduce the amount of viral shedding, so that there is less virus circulating through the herd.[3,9,10] One problem that arose from the use of PR vaccines was how to determine if an animal that tested seropositive for PR antibodies was positive due to infection with field virus or due to vaccination with a PR vaccine. In the past, serum-virus neutralization (SVN) antibody titers have been used to determine the infection status of pigs. The use of these antibody titers to determine PR status has proven to be somewhat difficult as some pigs that have been vaccinated multiple times may develop relatively high antibody titers. In addition, pigs that are in the early stage of infection may have no antibody titer or low antibody titers.

Recently, vaccines have been developed that contain gene deletions which can be utilized to differentiate vaccinated pigs from infected pigs.[6,7,11] The genes that have been deleted from the vaccines, and are used for diagnostic purposes, all code for glycoproteins that are present in the PRV envelope. In a gene deleted vaccine, the glycoprotein coded for by the missing gene is not produced by the vaccinated pig. Consequently, antibody directed against that glycoprotein is not produced due to the absence of the glycoprotein antigen.

Current technology has allowed for the development of diagnostic serology tests that are able to detect antibodies directed against a specific glycoprotein. These enzyme-linked immunosorbent assays (ELISA) have become popular tests to utilize in high volume laboratories because they are relatively quick and easily automated. Another advantage of the ELISA tests is that they are sensitive and specific and can detect antibody levels five to six days post-exposure to PRV.[1,8,12] An animal vaccinated with a gene deleted vaccine will test negative (no glycoprotein antibody present) on the companion diagnostic test, while an animal exposed to field virus or vaccinated with a PR vaccine containing that glycoprotein will test positive. One area of concern with gene deleted vaccines is the potential for field strains to be deficient in these same glycoproteins. The gI antigen has been extensively studied and, so far, over 250 strains of PRV from several continents have been examined for the presence of the gI antigen.[14] In every instance, gI has been present.[14] Currently, there are several differentiable vaccines that are available in the United States and have a companion diagnostic test (Table 1).

SV/PRV is an experimental MLV pseudorabies vaccine that has been developed for use in swine. It has been found to be safe for use in gestating swine and pigs as young as three days of age. In addition, the vaccine has been shown to reduce the severity of disease when pigs are vaccinated intranasally or intramuscularly and then are challenged with PRV. In addition to protecting against challenge the vaccine has been found to reduce the amount of virus shed by subsequently infected pigs. Deletions of the thymidine kinase gene as well as a portion of the internal inverted repeat region have been made to reduce the virulence of the vaccine virus. Deletions of the gpI and

gpX genes have been made in order to differentiate vaccinated pigs from swine that have been exposed to field virus.

Table 1. Licensed Gene Deleted Vaccines and Companion Diagnostic Test

Vaccine	Manufacturer	Diagnostic Deletion	Diagnostic Test
Bio-Ceutic® PRV	Boehringer Ingelheim	gpI	HerdChek® Anti-PRV-gI
PR-Vac®	SmithKline Beecham	gpI	ClinEase-PRV® HerdChek ® Anti-PRV-gI
PRV/Marker®	SyntroVet, Inc.	gpX	HerdChek® Anti-PRV-gpX
Tolvid®	The Upjohn Co.	gpX	Tolvid Diagnostic®
OmniMark™	Fermenta Animal Health	gpIII	DiaSystems™ CELISA OmniMark™ PRV

MATERIALS AND METHODS

General

Animals and housing

All pigs were obtained from pseudorabies free herds and were housed in secured facilities. Pigs were bled at the time they were placed in isolation facilities.

Virus

The virus strain used in the challenge experiment was the pneumotropic strain VDL 4892. This virus was grown on Madin-Darby bovine kidney (MDBK) cells, harvested, and stored at -70°C until needed. The challenge virus was titered at the time pigs were challenged. This was accomplished by making ten fold dilutions of the challenge virus. Eight wells of each dilution containing 50 µl of virus and 150 µl of MDBK cells were incubated at 37°C for 48 hours and then examined for typical cytopathic changes. The titer was determined using the Kärber method.[13] The virus was given intranasally using a 3 cc syringe which was fitted with a 16 gauge needle that had been shortened to approximately 5 mm in length. Rubber tubing[a] of size 1.6 mm X 0.8 mm was cut into a 35 mm piece and placed over the cut needle.

Vaccines

PRV/Marker® and the experimental vaccine SV/PRV are both MLV vaccines and were supplied by SyntroVet, Inc.[b] SmithKline Beecham's gpI

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[a] Fischer Scientific Company, Eden Prairie, Minnesota.
[b] SyntroVet, Incorporated, Lenexa, Kansas.

deleted vaccine PR-Vac®[c] and Boehringer Ingelheim's gpI deleted vaccine Bio-Ceutic® PRV[d] are both MLV vaccines and were purchased through a local vaccine supplier.

Serology

Serum samples were tested with commercially available PRV ELISA kits. These kits included the HerdChek® Anti-PRV Screen test[e], which is designed for use in nonvaccinated pigs, the HerdChek® Anti-PRV-gpX test[e] for pigs vaccinated with PRV/Marker or SV/PRV, the HerdChek® Anti-PRV-gI test[e] for animals vaccinated with Bio-Ceutic, PR-Vac, or SV/PRV, and the the ClinEase-PRV test[f] for animals vaccinated with PR-Vac or SV/PRV. All day zero serum samples were tested on the screening ELISA test to establish that the pigs were seronegative for PRV antibodies. Antibody titers were determined using a slightly modified version of the serum-virus neutralization (SVN) test that is described by Hill et al.[5]

Trial 1: Multiple vaccination with SV/PRV

Ten pigs were vaccinated with a 10X dose of SV/PRV intramuscularly on days 0 and 20. A 1X dose was given intranasally on day 72. The pigs were challenged with a 1 ml dose ($4.7X10^6$ $TCID_{50}$/ml) of VDL 4892 pseudorabies virus on day 98. Each pig received 0.5 ml/nostril. Pigs were observed for fourteen days after challenge. All pigs were bled on days 20, 29, 42, 72, 81, 98, and 112 post-vaccination.

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[c] SmithKline Beecham, Lincoln, Nebraska.
[d] Boehringer Ingleheim, St. Joseph, Missouri.
[e] IDEXX, Incorporated, Portland, Maine.
[f] SmithKline Beecham, Lincoln, Nebraska.

Trial 2: Licensed vaccines followed by vaccination with SV/PRV

Twenty-five pigs (no. 1R-25R) were vaccinated intramuscularly with PR-Vac according to the manufacturer's directions, and twenty-five pigs (no. 1Y-25Y) were vaccinated intramuscularly with PRV/Marker according to the manufacturer's directions. Twenty-two days post-vaccination, the pigs from both groups were bled and vaccinated intramuscularly with a standard 2 ml dose of SV/PRV. All of the pigs were bled on day 37.

Twelve pigs (no. 101-110) were vaccinated intramuscularly with a 10X dose of Bio-Ceutic on days 0, 9, 22, and 36. A 10X dose of PR-Vac was given intramuscularly on days 53 and 65. A 1X dose of SV/PRV was given intramuscularly on day 73. The twelve pigs were bled on days 9, 73, and 82.

RESULTS

Trial 1: Multiple vaccination with SV/PRV

All ten pigs remained seronegative to the ClinEase gI, HerdChek gI, and HerdChek gpX tests until the time of challenge. All pigs were seronegative on the SVN test on day 20 except for pigs 101 and 103, which had titers of 1:2. The pigs remained clinically normal throughout the fourteen day observation period post-challenge. By day 14 post-challenge, the pigs were seropositive on all three differential tests and showed a rise in antibody titer on the SVN test (Table 2).

Table 2. Serum-virus neutralization antibody titers

Pig No.	Day 29	Day 42	Day 72	Day 81	Day 98 (CH)[1]	Day 112 (14PC)[2]
101	64	32	2	2	<2	512
102	8	4	2	<2	2	256
103	32	8	2	2	2	256
104	32	ND[3]	2	<2	2	2048
105	64	8	2	2	<2	2048
106	16	8	2	2	2	256
107	16	8	2	<2	<2	256
108	16	ND	2	4	<2	256
109	32	16	4	4	<2	512
110	16	8	2	<2	<2	512

[1]CH=day of challenge.
[2]PC=days post-challenge.
[3]ND=not determined due to insufficient serum quantity.

Trial 2: Licensed vaccines followed by vaccination with SV/PRV

Twenty-four of the PRV/Marker vaccinated pigs and twenty-three of the PR-Vac vaccinated pigs were followed to the end of the study. The remaining three pigs could not be evaluated because two of them died (no. 3R and 24Y) and one lost an ear tag (no. 6R). The PRV/Marker vaccinated pigs were seronegative on the gpX test following vaccination with the PRV/Marker vaccine and following vaccination with the SV/PRV vaccine. The PR-Vac pigs were seronegative on both gI tests following vaccination with PR-Vac and following vaccination with SV/PRV. The SVN antibody titers were 1:2 or less on day 22 and 1:16 or less on day 37 post-vaccination.The pigs vaccinated with Bio-Ceutic, followed by PR-Vac, and SV/PRV remained seronegative on the HerdChek gI test. The antibody titers are shown in Table 3.

Table 3. Serum-virus neutralization antibody titers for pigs vaccinated with Bio-Ceutic, PR-Vac, and SV/PRV.

Pig No.	Day 9	Day 73	Day 82
91	<2	8	8
92	<2	8	4
93	<2	8	8
94	<2	4	8
95	<2	4	8
96	<2	4	8
97	<2	4	4
98	<2	8	8
99	<2	8	8
100	<2	4	8
111	<2	8	4
112	<2	8	16

DISCUSSION

Trial 1: Multiple vaccination with SV/PRV

The multiple vaccinated pigs developed moderately high SVN antibody titers by 29 days after the initial 10X dose of vaccine. The antibody titers were still elevated after a second 10X dose of vaccine, but the antibody titers were actually lower after the second dose compared to the first dose. In addition, serum samples collected nine and twenty-six days after the intranasal vaccination revealed that the SVN antibody titers had dropped to very low levels and some of the pigs were serologically negative on the SVN test. The pigs were able to produce higher levels of neutralizing antibodies, as was seen when the antibody titers rose fourteen days post-challenge. It is not understood why the antibody titers decreased so rapidly, especially after booster vaccinations were given. The serum samples were placed into plastic tubes at the time of collection and immediately frozen until they were tested at the end of the trial. Each tube was identified with trial number, date of collection, and day of the study, and each bleeding was kept in a separate box. The samples were all tested at the end of the trial to alleviate the problem of day to day test variation. The samples were retested and the same decrease in antibody titer was seen on the second test. In addition, the same decrease in antibody titer was seen when the samples were tested by another laboratory. The decrease in antibody titer is important to consider from a diagnostic point of view, but from a clinical point of view, the animals responded to challenge by producing high levels of antibodies and they did not develop clinical signs of PR following challenge.

Multiple vaccinations, route of inoculation, and large doses of vaccine virus did not affect the serology results on the gpX and both gI differential tests. All of the pigs evaluated remained seronegative on each of the tests until they were challenged with PRV. Following challenge, all of the pigs seroconverted to a positive status on the three differential tests. As long as pigs are not exposed to a source of gI or gpX, such as through exposure to PRV, gI, or gpX containing vaccines, multiple vaccinated pigs will remain seronegative on the differential tests.

Trial 2: Licensed vaccines followed by vaccination with SV/PRV

SV/PRV was evaluated for its diagnostic compatibility with one gpX and two gI deleted vaccines that are currently licensed in the United States. In each instance, the pigs vaccinated with a gpX deleted vaccine remained seronegative on the gpX test and the pigs vaccinated with one or both gI deleted vaccines remained seronegative on the gI tests following vaccination with SV/PRV. This indicates that the vaccine can be used in herds already vaccinated with one of these vaccines without noninfected pigs seroconverting on the corresponding differential test.

The large number of pseudorabies vaccines currently on the market has led to a problem with the diagnostic testing of vaccinated pigs. Many producers do not keep good records in regards to whether pigs have been vaccinated, and if they have been vaccinated, which vaccine has been used. Some producers have incorrectly or inadvertently vaccinated with one glycoprotein deleted vaccine and then have given a follow up vaccination using a vaccine that contained the glycoprotein that was absent in the first vaccine. In addition,

many pigs are purchased without knowledge of the vaccination status of the pig. This has led to problems with animals appearing to be field infected when in fact they are seropositive due to vaccination.

The availability of SV/PRV in the market place should help to alleviate some of the problems associated with diagnostic evaluation of pigs when a producer wishes to change from a gpX or gI deleted vaccine to the double deleted SV/PRV. Prior to the development of this vaccine, there was not an alternative vaccine available that could be used in an animal already vaccinated with a gI or gpX deleted vaccine without causing some diagnostic confusion. There will still be problems requiring testing samples on several differential tests due to incomplete or inaccurate producer records, but it is hoped that this vaccine will reduce the problems associated with the changing of the vaccine that is used in a herd.

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Summary and Discussion

SV/PRV is a new generation of genetically engineered PR vaccines. This vaccine is unique in having two diagnostic genes deleted from the genome and two companion diagnostic tests. There is currently one other licensed vaccine in the United States which has two diagnostic genes deleted from the genome. This vaccine is licensed for use with a single companion diagnostic test. The main advantage of the two diagnostic tests is that SV/PRV can be used in pigs that have previously been vaccinated with the gI deleted vaccines Bio-Ceutic PRV or PR-Vac, or the gpX deleted vaccine PRV/Marker. If SV/PRV were licensed for use with only one of the companion diagnostic tests, the versatility of the vaccine would be significantly reduced.

One of the problems that arises with the use of two companion diagnostic tests is how to determine the PRV status of a pig when conflicting results are obtained from the two different tests. In a diagnostic laboratory, it is not uncommon to test serum samples for PRV antibodies using several different diagnostic tests because the swine producer does not know the vaccination status of purchased pigs, or because the producer has incomplete vaccination records. In a situation such as this, serum samples could conceivably be tested on both tests if the samples are positive on the initial test. A portion of the evaluation of the SV/PRV vaccine involved determining the seropositive status of known infected pigs on both the HerdChek gI and HerdChek gpX tests. In comparing the test results, it has became evident that fewer positive pigs are detected on the gpX test compared to the gI test. It is not known if this discrepancy is due to the mechanics of the test, properties of the gpX antigen,

or due to some other unknown factor. The important aspect of this information is that a discrepancy may exist. Should SV/PRV be licensed, consideration should be given to recommending that pigs vaccinated with only SV/PRV be tested on the gI test, and reserve the gpX test for those pigs which have been vaccinated with PRV/Marker and SV/PRV.

It is important to remember that the PR status of a herd is not based on the test results of a single pig tested at one point in time, but rather is based on many different pigs bled over a period of time. This reduces the risk of herds being released from quarantine when positive pigs have been incorrectly identified as negative on the serological tests. In addition, it is important to consider discrepant results in the context of herd history of PRV infection, reason for testing, and results of pigs tested at the same time. A single discrepant result in a case from a qualified negative herd which has no history of PR is going to be interpreted differently from a single discrepant result from a herd with some pigs which are seropositive on both tests. If these concepts are kept in mind, the infrequent discrepant results should not be a problem to interpret.

SV/PRV was shown to be safe when given intravenously to pregnant gilts and intracerebrally to three day old pigs. In addition, when given intranasally or intramuscularly, the vaccine was shown to reduce the clinical disease of PRV infection, length of time virus was shed from nasal secretions, and amount of virus that was shed compared to infected, nonvaccinated pigs. Based on clinical disease, duration of viral shedding, and amount of virus shed post-challenge, the efficacy of SV/PRV was comparable to PRV/Marker and PR-Vac when given intramuscularly. Pigs vaccinated multiple times with

SV/PRV were shown to maintain a seronegative status on both companion diagnostic tests. This is especially important for herds which maintain breeding stock for long periods of time, and which are vaccinated multiple times during the lifetime of the pigs.

The advantage of SV/PRV over the gene deleted PR vaccines that are currently licensed is the versatility of the vaccine. It was shown that pigs vaccinated previously with Bio-Ceutic, PR-Vac, or PRV/Marker maintained a seronegative status on the respective companion diagnostic test when vaccinated with SV/PRV. A review of the literature did not reveal any published information on the diagnostic compatibility of other licensed gene deleted vaccines. Therefore, SV/PRV has a distinct advantage over the other gene deleted PR vaccines in that is has been shown to be diagnostically compatible with Bio-Ceutic, PR-Vac, and PRV/Marker. This versatility gives the swine producer more flexibility in purchasing vaccinated pigs and in making the transition from one PR vaccine to another PR vaccine.

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