Whole herd testing in the Carroll County Pseudorabies Project: Its implications for the pseudorabies eradication effort in Iowa

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Departments: Veterinary Clinical Sciences
Microbiology, Immunology, and Preventive Medicine
Co-majors: Veterinary Clinical Sciences
Veterinary Preventive Medicine

Approved:

For the Graduate College

Iowa State University
Ames, Iowa
1992

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GENERAL INTRODUCTION

Pseudorabies has been present in the swine population of the United States for more than a century. With the advent of modern animal husbandry methods and improved disease diagnostic techniques the disease has grown to prominence in today's swine industry.

The United States is currently engaged in a nationwide effort to eradicate pseudorabies from the swine population. This effort is guided by the United States Department of Agriculture Pseudorabies Eradication State-Federal-Industry Program Standards; a document that outlines the procedures and steps of the national eradication program. According to these program standards, states are to be testing their swine herds on the basis of detecting, with 95% confidence, a 10% or greater intraherd pseudorabies prevalence. Iowa has chosen to continue to follow its eradication program guidelines, which were in place before the national eradication effort began. These guidelines allowed testing at a 95% confidence level of detecting a 20% or greater intraherd prevalence.

Validation of this testing level in Iowa swine herds is essential to the successful progression of the state eradication program.

Explanation of format

The following thesis consists of two papers. The first paper is a review of the initiation and procedures of the pseudorabies eradication program. The second paper presents the Carroll County Pseudorabies Project Whole Herd Testing study with its implications regarding the eradication effort in Iowa. Following the second paper is a general summary.

PAPER I.

REVIEW OF THE DEVELOPMENT OF THE PROGRAM FOR THE ERADICATION OF PSEUDORABIES IN SWINE

INTRODUCTION

Pseudorabies was described in the United States as early as 1813. Initially labelled as a "mad itch" in cattle of the Atlantic seaboard and the midwest, the typical syndrome included intense pruritus of the head or legs, bellowing, hyperexcitability, and death.[1] Cattle during this time were commonly grazed with pigs in cornfields. It was believed cattle contracted the disease by eating cornstalks that had already been chewed by pigs.

The term pseudorabies dates to Switzerland in the mid-1800s when the disease was described as being similar to clinical rabies, causing salivation, drooling, paralysis, and death.[2] Several species of animals were shown to develop the disease after inoculation with brain tissues from an affected bull and dog by a Hungarian physician named Aujeszky. Because of this work the syndrome was named Aujeszky's disease, the name still favored in Europe. The more descriptive, pseudorabies, is used primarily in the United States.

The emergence of pseudorabies as a primary disease in swine has resulted from the evolution of our animal husbandry practices and veterinary diagnostic capabilities. The growing popularity of confinement housing, often keeping more than one age of pig within a common airspace, has readily aided viral transmission. Paralleling this development was the introduction of more technically advanced methods of viral diagnosis such as the electron microscope and improved viral culturing techniques. This explosive transmission and more accurate diagnostic capability have placed pseudorabies among the more prominent disease of swine.

Pseudorabies virus

Description

Pseudorabies (PR) virus is a member of the viral family Herpesviridae and the subfamily *alphaherpesvirinae*. As with other members of this family, PR virus contains a core of linear double stranded DNA, an icosohedral capsid, and an envelope containing viral glycoprotein spikes on its surface.[3] Subfamily characteristics include a broad host range (all common farm animals but not humans and some species of nonhuman primates), efficient destruction of infected cells, and the ability to establish latent infections.

Host range

Swine are the only natural host and the primary reservoir in nature. They are unique among the species susceptible to the virus in their ability to become subclinically infected and to maintain latent infections. Latent infections can become established in various cells of the body, most notably in the trigeminal nerve ganglia of the brain, cells of the spinal cord, thymus, lymph nodes, and bone marrow.[4] During periods of stress or immunosuppression, reactivation may occur and result in viral shedding even with the absence of clinical signs. This phenomenon has been demonstrated by induction of immunosuppression with injection of dexamethasone[5], as well as by natural stressors such as parturition in the sow.[6] The level of viral shedding from the stressed latent carrier has been shown to be of a quantity adequate to cause infection in contact pigs.[7]

Viral genes and vaccine development

Viral virulence and immunogenicity are characteristics controlled by proteins produced

under the direction of the viral genome. Glycosylated envelope proteins identified as gII, gIII, and gp50 are primarily responsible for induction of an immune response to the virus.[8] While multiple genes control virulence, those encoding for the glycoproteins known as gI, gIII, gp63, as well as the thymidine kinase (TK) enzyme appear to be the most important in virulence expression.

The culture of viral strains genetically engineered to be deficient in one or more of the gI, gIII, gp63, gX, and TK encoding genes has demonstrated the ability of these strains to replicate without these genes being present. This has allowed the production of vaccines resulting in immunity that is protective from the development of clinical signs although not capable of preventing infection and the latent carrier state. This immune response is also differentiable from immunity produced by exposure to the wild PR virus through the use of monoclonal antibodies in enzyme linked immunosorbant assays. An immune response resulting in the production of antibodies to proteins that are absent in the gene-deleted vaccine virus is considered to have been caused by the wild virus. This differentiation allows the use of gene-deleted vaccine in the initial stages of the eradication program. Because of the ability of PR virus to produce latent infections, pigs with antibodies to proteins absent in the gene-deleted vaccine virus are assumed to be infected and asymptomatic carriers of the wild virus.

Clinical features

The clinical features of PR virus infection in swine vary with the characteristics of the infected population. Even though vaccination does not preclude infection it can prevent the

development of clinical symptoms. Thus, in a vaccinated herd an active infection may be inapparent. Nonimmune herds are most vulnerable to developing clinical disease after infection.

Seronegative pregnant females, if infected before 30 days of gestation, may resorb the embryos killed by the virus. Decreased herd fertility may be the result. Infection later in gestation may result in either abortion or delivery, at term, of pigs ranging from normal to weak, stillborn, macerated, or mummified. With rapid transmission from an index case or a carrier up to 50% of susceptible sows may abort.[9]

The mortality rate in pigs born to nonimmune sows may approach 100%. The incubation period is about 30 hours with the course of the disease being four to eight days. Fever, sneezing, coughing, constipation, and vomiting are initial signs that may go unnoticed. Clinical signs often first noted by the swine producer include muscle tremors, listlessness, incoordination, convulsions, and inability to rise although these symptoms usually don't occur until after 96 hours of infection. Pigs may become moribund and die shortly thereafter.

Colostral antibody, in vaccinated or recovered sows, may be in sufficient quantity as to be protective. Lasting eight to 12 weeks in the offspring, this maternal protection may be adequate to prevent high mortality rates. Following the decay of the maternal antibody, titer infection may not necessarily lead to fatal disease. Generally, as the pig ages, a relative resistance to clinical signs and mortality develops, such that in mature swine the mortality rate is usually less that 2%.

Immunosuppression

The role of PR virus causing continuing financial losses to the swine producer is empirically evident but scientifically controversial. Studies have examined the effects of endemic PR virus in relation to presence of other diseases, including *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* pneumonia and streptococcal septicemia.[10,13] Insidious losses are often difficult to document due to varied swine husbandry facilities and practices.

In one attempt to investigate this question, six large swine herds in Illinois (containing more than 400 sows each) were serologically sampled for antibodies to PR virus, swine influenza, encephalomyocarditis virus, transmissible gastroenteritis virus, *Actinobacillus pleuropneumoniae*, *Eperythrozoon suis*, and six serovars of *Leptospira interrogans* (*bratislava*, *pomona*, *hardjo*, *canicola*, *grippotyphosa*, and *icterohaemorrhagiae*). The association between seroprevalence of PR virus and seropositivity to the other disease agents was examined. No evidence was found to support the assertion that endemic PR viral infection increases the risk of other bacterial and viral infections.[10]

Other workers have shown PR virus does have the capability of affecting the normal immune response in the pig. Down-modulation of the expression of major histocompatibility complex class I (MHC I) surface antigen expression in porcine cells by PR virus has been shown.[11] Surface expression of this antigen is directly related to the ability of the host immune system to recognize and react to viral infection. PR viral infection decreased MHC I expression by 60% or more in cultured porcine cells. PR viral infection has also been shown to decrease lymphocyte proliferative response and interleukin - 2 production in

experimentally exposed seven week old pigs.[12] The likelihood is that the virus has the capability of diminishing the host pig immune response to challenge by other infectious agents which may lead to infection with secondary bacteria such as *Pasteurella multocida* species. This would lead to increased production costs through increased use of medications as well as decreased feed efficiency and decreased rate of gain.

This position has been supported by an experiment in which seven week old pigs were experimentally inoculated with varying doses of PR virus and *Pasteurella multocida.* The dose of PR virus used for inoculation was directly related to the observed decrease in feed consumption and rate of gain as well as an increase in *Pasteurella multocida* pneumonic lesions.[13] Additionally, 104 Minnesota swine herds quarantined for PR virus infection were tested for seropositivity to *Actinobacillus pleuropneumoniae.* The presence of antibodies to both agents was positively correlated both on an individual pig and a herd basis. Whether the elimination of PR virus would facilitate removal of *A. pleuropneumonia* or removal of *A. pleuropneumonia* would facilitate PR virus elimination was not addressed.

Economic impact

Evidence of the economic impact PR virus has on swine operations is provided by a study analyzing financial and production records of 77 Illinois swine producers.[15] Using a three year average, a projected net annual return for a 120 sow herd without endemic PR virus was calculated to be $86,218. With PR virus projected to have a high impact on the swine herd productivity, the net annual return falls to $77,957. At a moderate PR virus level a decrease to $84,227 in net annual return is projected. Calculating total costs of PR virus

across all levels of influence within the herds (low, moderate, high), PR viral infection costs an average of $22.66 per sow per year.[15]

Estimations of clinical PR virus disease costs were developed, as well, by using disease rates reported through a survey of state veterinarians, information from the United States Department of Agriculture, and data gathered from pilot PR virus eradication projects. These projects were carried out in five states with the purpose of gathering data on prevalence and clinical costs of PR virus infection from the areas under study.[16] Data generated resulted in clinical disease cost estimates (in 1984 dollars) of $36.00 per sow per year for a farrow to finish producer, $22.00 per sow per year for a feeder pig producer, and $110.00 per sow per year for a seed stock producer. This yields an average estimated nationwide 1984 annual cost of $6,623,726 due to clinical PR viral disease, and a total estimated PR viral annual cost to the U.S. swine industry of $32,918,376.[16] Other estimates of mortality and abortion losses from PR viral infection go as high as $21.4 million to $25.6 million annually when costs of vaccination are included.[17]

NATIONAL PSEUDORABIES ERADICATION PROGRAM

Prevalence of pseudorabies

With an increased awareness of PR virus as an economically important disease in swine, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) conducted four national serological surveys of slaughter swine, between the years 1974 and 1984. Monitoring the prevalence of PR virus in the nation's swine population via serologically sampling slaughter swine was the goal (Table I).[18]

Table I points to a rise in market hog seropositivity during the latter years of the 1970s and, on a nationwide basis, a leveling off during the early 1980s. The dramatic rise seen between the 1977-8 and the 1980-1 samplings is partially due to the increased use of

Table I Serological survey on slaughter swine (percent of market swine positive by serum neutralization test)

	1974	1977-8	1980-1	1983-4	
				breeders	mkt hogs
Total U.S.	0.56%	3.73%	8.39%	18.80%	8.18%
Pilot Project States					
Illinois	1.14	3.29	6.40	17.05	6.75
Iowa	0.55	5.82	13.04	34.29	14.14
North Carolina	0.00	3.44	6.45	0.00	5.87
Pennsylvania	2.20	1.59	10.53	6.25	5.21
Wisconsin	0.52	1.41	2.96	4.76	1.74

undifferentiable PR viral vaccines, however, increasing seroprevalence in the heavily populated swine states of Illinois and Iowa is evident both before and after the 1980-1 introduction of vaccination.

Pseudorabies eradication pilot projects

As a result of these findings the National Pork Producers Council (NPPC) and the Livestock Conservation Institute approached APHIS suggesting the initiation of five pilot eradication projects designed to test the feasibility of eradicating PR virus in swine herds within a limited geographical area. Marshall county, Iowa, Pike and Macoupin counties, Illinois, and the states of North Carolina, Pennsylvania, and Wisconsin were selected to participate in this effort.[18]

As a result of these pilot projects the following are among conclusions reached:[18]

1. 97.5% of the herds found to be infected during the first 18 months of the 3 year pilot project were successful in removing PR virus from the herd.

2. PR virus eradication programs work best with widespread herd owner participation, thus allowing application of an eradication program on an area wide basis.

3. Surveillance via on farm testing was successful in areas of high prevalence, while trace back of positive serum samples from slaughter surveys proved to be difficult. This on farm testing was successful when serological surveys were done by a statistical sampling that would yield a 95% probability of detecting infection in herds in which at least 10% of the breeding stock are seropositive.

A financial feasibility analysis of continuing with a national PR virus eradication

program determined a cost:benefit ratio with a range of $1.3 million:$4.2 million (1:3.2) to $1.1 million:$2.1 million (1:1.9) over the ten year period of a proposed eradication program.[16] The pilot project successes and this financial analysis gave impetus to the NPPC to endorse a statement supporting the eradication of PR virus from the swine herds of the United States in 1987. January 1, 1989 was the beginning of the national pseudorabies eradication program.[19]

PROGRAM STANDARDS FOR PSEUDORABIES ERADICATION

The national eradication effort is guided by a pseudorabies eradication program standards document published by the USDA-APHIS. The program standards outlined in this text were developed by the United States Animal Health Association as minimum standards designed to lead to eradication of PR virus from all domestic swine in the United States. Individual states have the option to adopt more stringent standards should the state elect to do such via the passage of specific legislation.

The state-federal-industry program standards manual outlines four parts to the PR virus eradication program. They are as follows[20]:

Part 1 Definitions

Titles, words, terms, and phrases pertinent to the PR virus eradication effort are defined.

Part 2 Administrative procedures

Regulations dealing with administration of the eradication program on the state and federal levels are outlined. Duties and powers of state and/or federal eradication program officials are detailed.

Part 3 Program stages and requirements

Within this section the qualifications and requirements of the steps through which states progress to PR virus free status are outlined.

It is imperative that one has a basic knowledge of the process through which states must pass to gain PR virus free status in order to understand how Iowa and the other major

pork producing states must relate to each other within the national eradication effort, therefore, the five eradication program stages are explained in the following:

Stage I

During this stage of initial entry into the eradication program the state wide procedures to control and then eradicate PR virus are documented.

This committee is made up of representatives from all segments of the state's swine industry. The pork producers and their organizations are represented. State government is represented by the officials of the state agencies charged with implementing the eradication program. Experts in PR virus epidemiology and additional interests associated with the state's swine industry, for example auction market representatives, are also included.

Means to determine area and state prevalence of PR virus in the state's swine population must be found and a plan for implementation must be formed. Required reporting of PR virus isolation or suspected case findings by producers, veterinarians, and diagnostic laboratories begin to shed light on the incidence of clinical PR virus infections within the state. Adding to the determination of overall prevalence is the collection and testing of serum samples from sows and boars at slaughter establishments and auction markets. Serum collected on the farm for purposes such as disease detection and diagnosis, exhibition requirements, brucellosis testing, and testing at the time of change of ownership are also analyzed.

The swine production industry and state officials must seek state legislation to allow regulation of suspected PR virus outbreaks by the necessary officials, conduct epidemiological

investigations, quarantine facilities infected with PR virus, and regulate intrastate and interstate shipment of swine, in addition to similar authorities needed for further PR virus control and eradication.

Swine industry educational efforts are a cornerstone of a successful control and eradication program and need to be developed and functioning on a state wide basis.

Stage II

The goal of the second stage in PR virus eradication is to gain control of the virus via active identification of infected herds, elimination of the virus from these herds and continued cooperation with the national program.

The state must document the implementation of Stage I standards. States newly entering this level must immediately initiate a surveillance program of circle testing around identified infected herds. States in stage II as of January 1, 1992 must initiate a circle testing program by January 1, 1993.

Tighter controls of swine shipments into the state are begun. Breeding stock, feeder pig and finisher pig movement is more closely regulated than in Stage I and must meet specific testing and documentation requirements.

Intrastate movement of swine is controlled to meet state needs and herd cleanup of infected herds by owners is on a voluntary basis.

Stage III

Requirements for entry into Stage III of the eradication program include the shift from voluntary to mandatory cleanup of infected herds. The PR virus status of the receiving and

source herds for swine movement is established. Prevalence of PR virus within all groups of swine, including finishing swine in feedlots, must be determined by continuing surveillance via serum samples collected at slaughter, at first points of concentration such as auction markets, and through down the road area testing. Vaccination may be permitted as part of an approved herd cleanup plan which is developed by cooperation among the swine producer, the herd veterinarian, and a state epidemiologist trained in PR virus epidemiology. Mandatory depopulation of herds with owners either unwilling or unable to carry out programs leading to PR virus elimination may be considered.

The swine industry must increase educational efforts and must state its commitment to the further advancement of the eradication program.

Finally, through the use of these identification and eradication procedures, the state must be able to demonstrate a prevalence of PR virus within its swine population of not more than one percent.

Stage IV

Stage IV is primarily a stage of continued breeding and finishing herd surveillance. Regulations controlling testing and swine movement into the Stage IV state as well as maintaining the surveillance standards began during Stage III of the program are implemented. A slaughter hog surveillance program including finishing swine from feedlots is also included.

Vaccination is permitted only as part of an approved herd cleanup plan or as designated by a state animal health official in a high risk area.

The Stage III surveillance program must have been in effect for at least two years. No known PR virus infection exists within the state and no new cases of infection could have occurred during the year prior to Stage IV application. An exception is infection traced to out of state importation, although this infection must not have resulted in spread to other premises.

Stage V

The final stage of the eradication program is that in which the state is declared PR virus free. The state must have had no PR virus infection for one year following recognition of Stage IV status and surveillance for the virus continues. No vaccination against PR virus is permitted, except by permit from the state veterinarian in cases of certain high risk herds.

Table II lists the states and their current PR virus national eradication program status.[21]

Part 4 Participation in herd plans and release from quarantine

The last part of the pseudorabies eradication program standards document details the establishment of individual herd PR virus status and methods approved for eliminating PR virus from a herd.

Qualified pseudorabies negative, qualified negative - gene altered vaccinated, pseudorabies monitored feeder pig herd, and pseudorabies monitored - gene altered vaccinated feeder pig herd are individual herd pseudorabies statuses gained via compliance with specific PR virus testing requirements, vaccination protocols, and herd management procedures. Random statistical serological sampling of a swine herd, as required under these guidelines, is designed to detect, with at least 95% confidence, a positive individual in a herd with a 10%

Table II Pseudorabies national eradication program status as of July 15, 1992

Stage I	Preparation		
Delaware	New Jersey	Iowa	Maryland
Pennsylvania	Oregon	Vermont	Rhode Island
Florida	Puerto Rico		
Stage II	Control		
Indiana	Illinois	California	Kentucky
Massachusetts	Kansas	Oklahoma	Nebraska
Idaho	Georgia	South Dakota	Washington
Tennessee	Missouri	Louisiana	
Stage II/III	Split state status		
North Carolina	Minnesota	Michigan	
Stage III	Mandatory herd clean up		
Virginia	Ohio	Mississippi	West Virginia
Wisconsin	Arizona	Arkansas	Alabama
Colorado	South Carolina	Montana	North Dakota
Texas	Nevada		
Stage IV	Surveillance		
Connecticut	Utah	New Hampshire	New York
Alaska	Hawaii	Wyoming	New Mexico
Stage V	Free		
Maine			

or higher intraherd prevalence. Table III details the number of individual swine needing to be tested according to the eradication program standards, which mandates testing on a level at least in compliance to this 95/10 measure. Table IV shows the level of testing required in large populations, essentially an extension of the 95/10 program standard requirements.[22] Table IV also lists the number of individuals needed to be tested to detect infection with 95% confidence at various other prevalence levels.

Table III Level of serological testing to satisfy the Pseudorabies Eradication State-Federal-Industry Program Standards, effective January 1, 1992

Number swine in breeding herd	Number swine to be tested
10 head	test all
11 - 35 head	test 10
36 or more head	test 30 percent or 30, whichever is less

The final subpart of Participation in Herd Plans and Release of Quarantine (Part 4), lists the methods by which a producer may gain quarantine release. These procedures warrant description, as they will directly affect the pork producer's enterprise and the PR virus eradication costs incurred before the realization of economic benefits.

Table IV Sample size required to detect reactors in a population of the stated size, at a 95 percent certainty level

For 95% certainty:

	Maximum permissible percentage reactors in group[a]								
Size of group	1%	2%	3%	4%	5%	10%	20%	30%	40%
100 or below	95	78	63	52	45	25	13	9	6
200	155	105	78	62	51	27	13	9	6
300	189	117	84	56	54	28	14	9	6
400	211	124	88	68	55	28	14	9	6
500	225	129	90	69	56	28	14	9	6
infinite	299	149	99	74	59	29	14	9	6

[a]Maximum permissible percentage reactors in group correlates with the actual intraherd prevalence

Methods of herd cleanup

Depopulation / Repopulation

This option removes all swine from the premises and the premises are to be cleaned, disinfected, and maintained free of swine for 30 days or a period of time determined adequate by an official pseudorabies epidemiologist.[20]

The time required to realize depopulation may vary with individual producer situations. With farrow to finish enterprises, depopulation most probably could not be completed for a period of months. Electing to completely depopulate the breeding herd without regard to stage of gestation or lactation would still leave feeder pigs and young finishing stock on the farm. Without facilities on an alternate site additional time, in months,

would be needed to allow the economic removal of this population segment. With phased breeding stock removal the time to depopulation would be dependent on the herd culling rate.

Once depopulation has been reached, premises disinfection may be started. Removal of all organic material from floors, feeders, waterers, etc. is needed to disinfect these objects effectively. Fomite protection of the virus and, therefore, transmission via exposure of susceptible swine to contaminated fomites is of concern. Although the virus is quickly inactivated when exposed to sunlight and drying conditions, infectious levels of PR virus have been found to be maintained as long as four days when the virus is suspended in swine nasal washings then sprayed on straw bedding, 18 days when sprayed on steel, and 36 days in shelled corn after spraying with a suspension using glucose saline as a vehicle.[23] Cleaning all surfaces by high pressure spraying, followed by wetting with orthophenolphenate compounds, 5% phenol, sodium hypochlorite, calcium hypochlorite, 2% sodium hydroxide, trisodium phosphate, or chlorhexidine will disinfect the premises.[24]

If possible, depopulation should be finished during summer months, allowing drying of dirt lots during warm temperatures. Tilling the outside lots exposing as much dirt as possible to the sun and its drying will help eliminate the virus.

Cleanup time, defined as the time from the beginning of depopulation to completed repopulation and recertification of a PR virus free herd, has been as short as 2 to 3 months with immediate depopulation and as long as 28 months with a phased depopulation program.[25] Generally, depopulation/repopulation will need six to eight months, depending on the economic requirements of the producer.[25]

Given adequate cleaning and disinfection, depopulation followed by repopulation with known PR virus free replacement stock is the most reliable of the methods approved to eliminate PR virus from premises. An additional advantage may be the elimination of other diseases or genetics that may decrease efficiency in production thus increasing costs and sacrificing potential profits. Careful consideration of the replacement stock source is of paramount importance.

Primary among the disadvantages of depopulation/repopulation for obtaining PR virus free status is the economic burden that may be experienced by the producer. Net cost to the producer in terms of the difference between selling price of stock and the purchase price of replacement stock would be determined by current market conditions and may affect the herd cleanup strategy. The major economic burden associated with depopulation/repopulation herd cleanup, however, is related to facility and production down time. Down time not only includes the time needed to allow facilities to sit empty (30 days) but also the time lost from production/sales before the operation can again begin realizing income from sales. In a farrowing situation five months or more may pass before the sale of the first finisher pigs following repopulation. This time represents lost income had the sale of pigs been proceeding as before the cleanup operation began. Estimates of expense due to depopulation/repopulation, $203.66 per sow in 1989 dollars, include the approximation that $106.60 (52%) will be due to inventory economic opportunity lost during down time of the operation.[26] An economic analysis, done in 1987 from farm production records of a 150 sow farrow to finish herd in Ohio experiencing a PR virus epizootic, found 87.6% of a total

outbreak cost of $48,175 was attributable to down time needed to clean up from the infection. Although not able to measure losses attributable to depressed grow/finish performance, decreased fertility in sows, and vaccine costs/benefits had the disease remained enzootic in the herd, it was estimated the disease could have remained enzootic on the farm for 22 years before the accumulated costs would have exceeded those of depopulation/repopulation.[27]

Test and removal

Test and removal involves serologically testing all swine present on the premise and immediately removing any individuals found to be positive for anti-PR virus antibodies. Testing is repeated in 30 days and, if any seropositive individuals are again identified they should be removed from the herd. If after four similar tests done at 30 day intervals seropositive individuals are still being identified, an alternative method of cleanup should be considered.[28]

Principal among the benefits of test and removal are the preservation of the genetics established in the herd, relatively short time needed for resolution of the imposed quarantine, and low economic commitment needed by the producer, assuming a stable breeding herd population is maintained during herd cleanup.

Preservation of the herd genetic base is primarily a factor of the type of swine operation under consideration. In a farrow to finish operation, preservation of the herd genetic background can be achieved through using the finishing unit gilt population as a source of replacement stock for the sows removed through the test and removal process. The ability to tap the finisher unit as a source of replacement females is dependent on the finisher

unit either initially being or first achieving PR virus negative status. Should either of these prove not to be attainable the genetic potential in the finisher unit would be unusable.

The economic impact of test and removal, versus other PR virus cleanup procedures, on farrow to finish operations has been examined for a group of swine herds in Iowa.[26] Using producer derived estimates of the value of removed and replacement stock, it was determined that, in five herds using this cleanup method, an average cost per sow in inventory was $7.78. This includes both direct producer costs and costs incurred within the state's PR virus cleanup program. Using the same analysis, however examining different farms, this cost compares with total cleanup costs of $203.66 per sow in using depopulation/repopulation and $40.87 per sow if removing PR virus via offspring segregation, a procedure to be discussed shortly. It should be noted that many factors, including original herd prevalence and market conditions, will affect the selection of a PR virus cleanup method and the incurred cleanup costs. In addition, the comparison of costs among cleanup methods cannot be definitively determined without taking into account variability between farms, since the same farm cannot serve as a trial for multiple cleanup methods.

In the situation of an operation producing feeder pigs and without a gilt pool being supplied by the farrowing operation, there is no opportunity for the herd internally to supply replacement individuals of like genetic makeup to those leaving the herd. In this scenario the maintenance of a herd genetic base has been, and would continue to be, dependent on the availability of PR virus negative replacement stock from a point source or a collection of specific sources.

PR virus cleanup of swine operations supplying "seed stock", males and females originating from specific genetic lines, designed to be sold from the farm of origin, and used as replacement stock in other swine operations, would necessitate conservation of genetic lines should the producer want to continue supplying similar stock. Maintenance of genetic lines and cleanup in a timely fashion are attractive advantages to test and removal for the seed stock producer.

In the case of a feeder pig finishing operation, test and removal would most probably not be a viable alternative because of the relatively short time period PR virus positive pigs would be on the premise. In such systems economics would dictate depopulation/repopulation as the more likely avenue of virus elimination, most probably in conjunction with vaccination and/or other management techniques maintaining pig movement through the unit while minimizing intraherd viral transmission.

Offspring segregation

Utilizing offspring segregation necessitates isolating offspring from the sow during that time of the pig's life in which it is most likely to be PR virus negative. This type of program is dependent upon the availability of facilities in which the offspring can be raised. No direct contact nor sharing of the same air space with the breeding herd can be allowed. The breeding herd would still contain the original PR virus stock and thus remain as a possible source of continuous infection. Serologically testing the offspring at 12 to 16 weeks of age to verify PR virus negative status is necessary to confirm the successful isolation of the offspring. Offspring segregation could be practiced by any type of swine operation with

separated farrowing and nursery facilities.

Two offspring segregation protocols can be used during cleanup.[28] Immediate offspring segregation involves weaning pigs from sows as early as possible (less than three to four weeks of age) when it is hoped maternal antibody passed to the pig prevents infection. These pigs are separated to a nursery facility isolated from other swine in terms of physical location and air space. Serologically positive 12 to 16 week old pigs would signal the immediate removal of the pen in which the seropositive individuals were identified. Subsequent testing and grouping of pigs would eventually yield a gilt pool with seronegative status. Alternatively, in a delayed offspring segregation program, the gilts would be kept on the original premises until those testing seronegative at 12 to 16 weeks of age are isolated into a separate facility. As before, retesting and grouping eventually yields a PR virus negative replacement gilt source.

With the introduction of gene deleted PR virus vaccines, enabling serological differentiation between vaccinated swine and swine exposed to wild PR virus, modifications of offspring segregation (as well as test and removal) have become possible. Vaccination does not totally suppressing virus excretion but it has been shown to result in a 100 to 1000 fold reduction in the amount of excreted virus following experimental challenge.[29] Consequently, prefarrowing vaccination of the sows should result in less virus excretion, less exposure to the offspring, and more seronegative individuals at 12 to 16 weeks of age.

The primary advantage of the offspring segregation system lies in the areas of maintaining genetic material within the herd by eventually using offspring as a seronegative

breeding stock replacement source and minimizing disruption to the normal pig flow through the system. The economic impact of this cleanup program is minimized because with this program breeding, gestation, farrowing, nursery, and, if present, finishing facilities maintain their economic usefulness to the operation during the cleanup time. Breeding herd turnover rate can be maintained and, by using a culling selection process at least partly based upon PR virus status, the prevalence within the breeding herd will decline. Vaccination with a gene deleted vaccine and the resultant decrease in viral shedding could further be utilized in conjunction with culling practices, to further decrease breeding herd prevalence and virus exposure to offspring.

Offspring segregation, with or without vaccination, has a disadvantage in that time to quarantine release is the longest of any of the cleanup procedures. During the pilot PR virus eradication programs offspring segregation was successful in eliminating PR virus from 28 of 32 swine herds.[25] However, it took an average of 16 months (range, 4 to 31 months) for cleanup to take place.[25] Eventually, during the eradication process, the protracted time needed to cleanup by this method will be unacceptable.

Another disadvantage to offspring segregation, at least for some producers, is the need for isolated facilities to house offspring. Should a facility suitable to use as an isolation nursery not be available one must be built or rented, adding to the cost of implementing such a program.

Selection of a program that will result in reaching PR virus elimination should be based on multiple factors. Among the factors are intraherd prevalence, type of operation,

available facilities, value of genetic lines already established in the herd, financial and management considerations, replacement stock availability, area PR virus prevalence, and endemic diseases present in the herd.[28] These factors must be considered for individual herds because each herd and each herd owner is unique, with unique needs and agendas.

Prevalence within the herd is one aspect that has received much attention. Using computerized decision tree analysis and simulation modeling a hypothetical 100 sow farrow to finish operation, with a given variety of specific conditions, was analyzed to determine the most economical program for PR virus eradication.[30] Under a variety of circumstances and prevalences depopulation/repopulation was found consistently not to be the most economic option for PR virus elimination. Given prevalence of less than or equal to 57%, test and removal was the most economically advantageous cleanup method. At prevalence rates greater than 57% vaccination of sows, with gradual herd cleanup via offspring segregation, was found to be optimal. As higher gross margins were assigned, test and removal became the program of choice at all prevalence rates. The assumption was made that only two or three serological herd samplings would be needed for cleanup. In cases in which the prevalence in the herd is high due to active viral shedding and infection, two or three herd tests during test and removal may not be sufficient to eliminate all the infected swine.

Intraherd prevalence rate also may be used as an indication of the level of viral circulation within the herd at the time of the serological sampling. PR virus relies on the existence of a susceptible host to maintain itself within a population. While this particular virus has the ability to remain dormant within nerve ganglia for long periods, in order for the

virus successfully to perpetuate its species, it must at some time be able to escape this latent state and pass on to another susceptible host. If it possesses the ability of establishing latency but not the ability to recrudesce the virus would survive only as long as the particular host carrying the latent infection lives. Therefore, the virus must be able to be transmitted to allow species survival. This transmission cycle includes the ability to reproduce in a host, to escape or be shed from that host, to survive for some period of time in the environment or fomite, and to enter and infect a new host. Immune status of the new host is an important determinant in completing the cycle.

Endemic PR virus will spread through a susceptible population, infecting a majority of pigs within that population. The outcome of this infection may follow one of three scenarios. The infection may overcome the host, causing sufficient pathological changes that death is the ultimate outcome. Such is the usual case with infections involving neonatal and juvenile pigs. However, before succumbing to the infection, the virus may be shed from these pigs in sufficient amount to cause infection of cohorts or other population segments. A second possible outcome is successful viral elimination from the host by an immune reaction of sufficient strength, duration, and specificity to prevent viral invasion of cells or arrest viral transmission between cells. In this instance the host would be free of the virus, but would have detectable antibody titer compatible with an immune response to presented wild virus epitopes. Establishment of a latent or carrier state, as is present with the third alternative, also is possible. Latent infection within nerve ganglia causes special considerations and complicates the detection of infected individuals necessary in a disease

eradication program. The latent period may be long enough to allow the antibody response resulting from initial infection to decay to a minimal level. Thus an animal may be harboring the virus but not allowing sufficient viral contact with the host immune system to stimulate more than minimal levels of circulating antibodies. Viral recrudescence from infected cells may be at such a low enough level that the pig is able to keep the viral infection from causing clinical disease. It is these swine that present the greatest dilemma in viral eradication. Infection of these individuals may not be serologically noticeable until the virus is finally presented to the host in sufficient amount as to illicite a measurable and rising antibody titer. With severely stressed or immunologically compromised pigs, the recrudescing virus may overwhelm the protective mechanisms of the body and establish an ongoing infection. This individual could serve as a source of shed virus to infect other susceptible swine within the population.

The decision regarding the appropriate method of herd cleanup also must consider the state of the infection within the population instead of only within individuals. If the infection is actively spreading within the population, testing for and removing seropositive individuals will not be adequate to identify all infected swine. Those that are newly infected and, thus, may potentially be a source of continuing infection within the herd, may not be detected because of the lag time between infection and measurable immune response. In this instance one would expect a rising intraherd prevalence and test and removal would prove fruitless until the spread of infection has diminished.

The use of gene deleted vaccine has been evaluated in conjunction with various

elimination procedures. With vaccine available for use, vaccination along with test and removal is preferred when intraherd prevalence is over 20%, but test and removal alone becomes economically favored when the prevalence rate drops to less than or equal to 20%.[30]

The type of operation and the facilities available for use also will help dictate herd eradication programs. The seed stock producer's needs may be different from that of the farrow to finish producer when considering the most economical cleanup program. Facilities available and their usefulness in rearing pigs in isolation needs to be kept in mind, as well.

As discussed earlier, value of the genetic background of the breeding stock may be a primary focus for individual producers. In an effort to maintain blood lines producers may consider the disadvantages of the cleanup programs such as test and removal and offspring segregation minor in relation to the advantage of maintaining the same genetic background in the breeding herd.

Management capabilities and financial status of the herd owner need to be considered when deciding upon a herd cleanup program. Producers not able to manage pig flow and traffic among various swine groups may not be good candidates for implementing offspring segregation, however, they also may not be financially able to survive the nonproductive downtime associated with depopulation/repopulation. The status of the market conditions at the time of herd cleanup affects the producer abilities to withstand herd PR virus eradication costs. Consultation with lenders and establishing a financial flow sheet may be necessary to arrive at an economically reasonable herd cleanup program and timetable.

In herds where concurrent disease problems add to production costs, for example by

death loss or increased feed costs, the benefit to be gained by depopulation/repopulation in terms of eliminating or minimizing these other diseases may outweigh its inherent disadvantages. Situations exist where PR virus may not be the primary cause of ongoing financial strain in the operation, however it may supply the impetus to enter a herd cleanup program that could yield additional benefits by decreasing the herd disease profile.

Finally, the prevalence of PR virus in the vicinity of the swine operation must be considered. As is the case with an individual herd, if there is active viral transmission among herds within an area, test and removal or depopulation/repopulation would most likely be expensive alternatives that may fail due to reintroduction of the virus to the newly negative herd. As removing a PR virus positive pig during a time of active infection within the herd and replacing that pig with one PR virus negative would most probably result in the newly introduced pig becoming infected, so would depopulation/repopulation of a swine operation most likely result in reinfection of that operation should the virus be actively spreading within its locale. Accordingly, without either an initial low area prevalence or an area wide effort timed such that the cleanup actions of individual herds would be conducted in concert, the recommendation of test and removal or depopulation/repopulation may not be advisable.

THE CARROLL COUNTY PSEUDORABIES PROJECT

Introduction

The swine producers of the nation have expressed their desire to eradicate PR virus from this country.[19] The successful PR virus eradication from the nation, state, county, or area is dependent on the same variables, such as economic concerns, prevalence, present state of the dynamics of PR virus infection, etc. that bear on the successful elimination of the virus from a herd. Extrapolating these variables from the individual, population, and farm level to the farm, area, and county level was the genesis of the Carroll County Pseudorabies Project (CCPP).

In order to keep farms from becoming reinfected, once they have successfully reached PR virus negative status, the prevalence within the area must be at a low enough level to allow detection and cleanup of infected farms before they can serve as a source for area infection. The CCPP was designed to help reduce the activity of the virus within swine populations resulting in decreasing prevalence within farms, areas, and finally counties of the state. The protocol was developed to yield decreasing prevalence within an area or county by decreasing the prevalence on individual farms. Decreasing interherd prevalence is as essential to a state wide eradication program as decreasing intraherd prevalence is to a herd eradication program. The CCPP strives to decrease intraherd and interherd prevalences as preparation for eradication, allowing the state's eradication effort to proceed as quickly and economically as possible.

PR vaccination of a swine breeding herd, as commonly done in the operations that

choose to utilize it, includes a primary vaccination before breeding and entry into the reproductive herd and booster vaccinations before each farrowing. With individual breeding herd females farrowing approximately twice yearly, this would result in approximately semi-annual vaccination of each breeding age female.

Protocol

The CCPP protocol uses the concept of reducing quantity and duration of viral shedding from hogs infected after vaccination to decrease intraherd exposure.[29] By increasing the frequency of vaccination it is hypothesized that the immune status of the individual can stay at a high enough level that, even if the pig in the herd sheds virus, the exposure to the susceptible population is held to a minimum.

By weaning pigs as early as possible, while passive antibody titers are protective, PR virus negative pigs begin to populate the nursery, grower, and finisher segments of the operation. Further, by operating these components on an all-in-all-out basis and by preventing the most likely positive and latently infected individuals (the sow breeding swine) from coming into contact with these pigs, the finisher barn should eventually reach seronegative status. Females could then be taken from this source and be used as seronegative breeding stock replacements.

Culling of sows from the breeding herd is carried out such that there is an average 50% annual culling rate. Many factors, among them being reproduction, production, and disease parameters, normally influence culling decisions. Including PR virus status among these criteria will result in increasing pressure on the virus within the herd due to the

elimination of potential viral shedding sources.

Additional production management procedures may augment the program. Isolation of swine groups, controlling pig movement on the farm to assist in maintaining isolation, and controlling traffic of people, animals, and machinery on the farm minimize the possibility of intraherd viral spread via mechanical, biological, or fomite transmission.

The best protocol aimed at preventing viral spread would be useless unless practical enough to be actually used by the swine producer within the swine production unit. The CCPP melds the best aspects of PR virus control into a production package that will cause a minimum of disruption, fitting within the normal operations of the swine unit. Whether continuous flow, all-in-all-out, PR virus positive, or PR virus negative each swine producer can implement some portion of the project protocol, thus applying pressure on the intraherd and interherd viral spread at minimized expense. The degree to which the herd is infected with PR virus, dynamics of the infection, the viral prevalence both within the herd and within the area, and the commitment the producer is willing to make toward the elimination effort, all impact on the implementation of the various components of the protocol and affect the success of the outcome.

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PAPER II.

THE CARROLL COUNTY PSEUDORABIES PROJECT WHOLE HERD TESTING; A PILOT PROJECT TO STUDY WHOLE HERD PSEUDORABIES PREVALENCE AND ITS IMPLICATIONS FOR ERADICATION

INTRODUCTION

The national pseudorabies virus (PRV) eradication program, supported by the National Pork Producers Council and directed by the United States Department of Agriculture, Animal and Plant Health Inspection Service, officially began January 1, 1989.[1] The purpose of this program is to detect infected swine herds, as identified by PRV seropositive status or clinical manifestations of the disease, confirm infection by laboratory procedures, and implement cleanup procedures within those herds that would ultimately result in herd, county, state, and national PRV free status.

Upon identification of a PRV positive swine herd within the state of Iowa, the swine producer may either (1) adopt a herd cleanup plan, including any or a combination of segregation of progeny, test and removal, or depopulation, (2) enter a feeder pig cooperator herd plan allowing movement of feeder pigs within the state if the farm has had 6 months with no clinical PRV signs, the pigs can be weaned at five weeks or less and reared segregated from the remainder of the herd and the producer has agreed to an approved PRV eradication plan, or (3) become quarantined, not being able to remove swine from the herd unless moved directly to slaughter or to an approved premises for finishing when accompanied by a certificate of inspection.[2]

All these options necessitate added costs of production that must be borne, at least in part, by the swine producer. Methods of herd cleanup and their economic impact on the swine enterprise have been well described.[2,3,4,5,6]

The use of gene deleted PRV vaccine has been shown to reduce both the amount and

duration of viral shedding following experimental challenge in swine, irrespective of the particular viral gene that has been deleted.[7] Consequently, vaccination has become a production procedure that may be useful in augmenting swine breeding herd PRV cleanup.

The Carroll County Pseudorabies Project (CCPP) was designed as a "producer friendly" program that would put pressure on endemic PRV with minimal disruption to the normal pig flow through a swine unit or the normal management practices of swine operations. Through the use of PRV vaccination in the breeding herd at least four times yearly, an annual herd turnover rate averaging 50% (based at least partly on PRV status), segregation of the finishing and breeding herds, and the control of pig, people, and mechanical traffic within and between farms, the prevalence of PRV within an area can be reduced in anticipation of the implementation of an eradication program by the state.[8]

National PRV eradication guidelines state that random statistical sampling of swine herds may be used to detect seropositive individuals and to establish the farm PRV status. This sampling is based on a statistical formula in which there is a 95% probability of detecting infection in a herd with at least 10% of the swine being seropositive. Each completely segregated group of swine on a premises is considered a separate herd for the purposes of PRV testing.[9]

The Iowa state PRV eradication pilot project determined that the average PRV infected swine breeding herd in Marshall county, Iowa, had a 57.5% intraherd prevalence.[10] On this basis, an Iowa swine herd is tested on the expectation of having a 95% probability of detecting a 20% PRV intraherd prevalence during a random statistical test. This correlates

to serologically testing 14 randomly selected breeding swine. Given the average of nearly 60% intraherd prevalence a PRV positive farm, as initially identified in Marshall county, there exists a wide margin of safety in this reasoning.

As a consequence of the utilization of the CCPP protocol on a farm, the prevalence of PRV infection, as determined by seropositivity, decreases.[8] The present study is designed, as a pilot study, to determine intraherd prevalence after two years of following the CCPP protocol and to determine if the sample size routinely used in Iowa's PRV eradication program is adequate to detect infection in these herds which have placed vaccine and culling pressures on the PR virus.

MATERIALS AND METHODS

Selection of participating herds

Swine producers in Carroll county, Iowa, participating in the CCPP for two years and completing the final random statistical serological sampling of their herd in a timely fashion were identified. Those herds in which this random statistical sample revealed the presence of a seronegative finishing herd and three or less seropositive breeding swine, within the 14 breeding swine tested, were invited to participate in the study.

Experimental design

CCPP participating swine producers meeting the selection criteria were identified, as were the Carroll county veterinarians servicing these herds. The veterinarians contacted their respective producers, asking if they would agree to have their entire breeding herd serologically tested for PRV.

Upon sampling, records for each individual hog in the breeding herd including identification via ear notch or permanent ear tag, age or parity, and PRV vaccination status, with the type of differential vaccine used in the herd, were developed.

The sera were tested using the appropriate enzyme linked immunosorbant assay for the particular type of vaccine used, yielding an intraherd PRV prevalence. This actual prevalence was then compared to the PRV serological results obtained by the random statistical sampling procedure and analyzed for correlation by SAS.

RESULTS

Nine of 15 Carroll county swine producers who met the criteria of the study elected to participate. The CCPP statistical sample testing was begun June 23, 1992 and the last whole herd test was completed September 30, 1992. Participating herd sizes ranged from 17 to 194 breeding swine, averaging 107 swine per breeding herd. Table I lists the herd sizes, results of the statistical sampling, and of the whole herd testing.

Data were analyzed for correlation using the SAS system. The size of the herd was statistically significantly correlated with the number of individuals found to be either seropositive or suspect during the whole herd test (Pearson Correlation Coefficient = 0.66, $p = 0.05$). Neither the actual prevalence found during whole herd testing nor the herd size had a statistically significant correlation with the percentage of tested animals identified as seropositive or suspect during the statistical sampling (Pearson Correlation Coefficient = 0.477, $p = 0.19$ for actual prevalence vs. statistical sample percent positive; Pearson Correlation Coefficient = 0.386, $p = 0.30$ for herd size vs. statistical sample percent positive).

Table I Results of the statistical sampling and whole herd testing done in Carroll county

FARM NUMBER	HERD SIZE	RANDOM STATISTICAL SAMPLING				WHOLE HERD TEST			
		PRV POS[a]	PRV SUS[b]	PRV NEG[c]	% POS[d]	PRV POS	PRV SUS	PRV NEG	% POS
1	101	1	0	27	3.6	2	4	95	5.9
2	78	2	0	12	14.3	21	2	55	29.5
3	17	1	0	12	7.7	2	0	15	28.6
4	173	0	1	13	7.1	4	12	157	9.2
5	114	1	0	13	7.1	2	0	112	1.8
6	84	1	0	13	7.1	9	2	73	13.0
7	108	1	0	13	7.1	24	0	84	22.2
8	194	3	0	11	21.5	54	12	128	34.0
9	90	2	0	12	14.3	0	0	90	0.0

[a]PRV seropositive
[b]PRV suspect
[c]PRV seronegative
[d]((number seropostive + number suspect) / total number tested) x 100%

DISCUSSION

The formula used to determine sample size needed to detect disease in a population is as follows:

$$n = \frac{\log P_b}{\log (1 - \textit{prevalence of disease})}$$

where n = sample size and P_b = the probability of a false negative result.[11] Thus, the size of the sample is dependent on both the confidence level assigned to the testing and the prevalence of the disease within the population. It is intuitively obvious that, as the prevalence of a disease within a population increases the number of samples needed to find at least one diseased individual decreases. Alternatively, as the confidence level of the sampling increases the sample size must increase.

Unlike the national PRV eradication standard, which tests herds at a 95/10 level (95% confidence of detecting at least one individual when the intraherd prevalence is at least 10%)[12] Iowa is currently conducting random statistical sampling at a level of 95/20, serologically sampling 14 swine from the resident breeding herd. During the initial stages of the eradication program Iowa policy has been to attempt to strike a practical and economic balance between the detection of PRV within its herds and the costs of the eradication program. With approximately 25% of the nation's swine population, the state of Iowa has particular interest in the eradication program. Testing at a 95/20 level, which was in effect before the program standard guidelines of 95/10 began, the state is attempting to maximize

testing of its swine herds while holding the state program costs down via testing fewer individuals in a particular herd but thereby being able to sample more herds. This position is not unreasonable given that, via statistical sampling, herds that were found to be serologically PRV positive during the Marshall county pilot project and the Carroll county PRV project had an average of 57%[10] and 62%[8], respectively, of the breeding herds PRV seropositive. Assuming, from these two projects, that a PRV positive herd is approximately 60% seropositive, one could use the equation determining sample size to find only 4 pigs in a herd would need to be tested to have a 95% probability of detecting the disease.

The results of this study, and the results of two separate projects carried out on swine herds in Minnesota,[12,13] point to the importance, however, of the need for some mechanism to be able to adjust sample size according to the prevalence of PRV within an area such as a county. In the instance of Carroll county, the testing of the complete herd gives an actual herd prevalence. Table I shows five of the nine herds were found to have a prevalence of less than 20% (range of 0% to 13%), which is the lower limit of prevalence detected in a 95/20 statistical test. This follows the completion of the CCPP protocol that is designed to assist the producers in decreasing intraherd prevalence. Since this is the first time such management procedures have been applied to a specific defined area on such an intense basis the exact affect on intraherd prevalence can only be hypothesized. Whether or not the CCPP procedures result in a significant decrease in prevalence on an interherd basis within the county, the effect of the project protocol on intraherd prevalence is of utmost interest. Whole herd testing was not done on any Carroll county farms prior to initiation of the project so the

before and after prevalence rates, unfortunately, cannot be directly compared.

It is reasonable to state, nonetheless, that in selecting for herds based on a seronegative finisher operation and an apparent low prevalence in the breeding herd, from 3 or less out of 14 pigs being seropositive, this pilot project was biased toward whole herd testing of herds most likely to have low prevalence. This is supported by Table I showing that, out of an average herd size of 107 breeding animals, 25 (23%) of these animals tested PRV seropositive. This is substantially lower than the estimate of a 62% prevalence rate within all the breeding herds of the county before the CCPP project began. Thus, if Iowa maintains the position of a 95/20 test as the eradication program progresses, in particular after CCPP like procedures are practiced on more farms in other areas, there exists the possibility of arriving at false negative results, on a farm basis, because of inadequate sample size to detect lower intraherd prevalence. A false sense of security would be devastating to the eradication effort.

A point of interest is that during statistical sampling of the nine experimental swine herds on a 95/20 basis, five herds had an intraherd prevalence rate of less than 20%. There are two likely explanations for this occurrence. This may speak to the fallacy of the basic assumption that swine herds sampled for PRV serology are always tested by random selection of individuals. Hogs may be selected on a practical rather than random basis. Gilts are easier to catch and hold than sows. Perhaps a particular pen of sows is closer or cleaner than another pen. These and other factors affect the psychology of assigning sows to the sample pool and may bias the results to a negative outcome, contrary to the actual herd PRV status.

Thus, instead of testing at a 95/20 level if pigs are randomly assigned to the tested group, by nonrandom assignment the test may be at some other confidence and prevalence level as determined by the selection of the pigs tested. Additionally, the nine herds participating in this study is a small portion of herds in the county. The small number of herds was necessitated by the costs of testing. This small sample size may make the results appear skewed. Should more herds be tested, the actual level of confidence and prevalence of the test sample may be correct. More herds need to be tested on a whole herd testing basis, also including herds that were classified as negative via statistical sampling, in order to validate how many actual PRV positive herds are being falsely called negative due to the present sampling parameters. Intraherd prevalence of herds with ranges of seropositivity could be established, thus acting as a guide in adjusting state policy from 95/20 testing to 95/10, 95/8, or even 95/5 during the latter stages of the eradication program. This would allow the program to proceed in a confident, scientifically sound, manner.

The view that the testing confidence/prevalence level need not be changed because of the assumption that, once the intraherd prevalence is below 10% or 20%, the virus is in a state of decline and would eventually be eliminated also could be presented. While it may be that such an opportunity exists, an alternative scenario has been explored. During one such examination 19 singlet PRV seropositive sows (an individual seropositive sow identified when sampling herds on a 95/10 sampling basis) were immunosuppressed, euthanized, and examined for PRV virus. One sow was PRV positive via virus isolation and four source farms of four other sows experienced PRV outbreaks after these singlets were identified.[14]

Controversy remains as to the role of the singlet reactor, however low prevalence should not be assumed always to extrapolate to eventual PRV negative status.

The presence of one herd in which two samples from the statistical testing were found to be seropositive and on retesting during the whole herd test were found to be seronegative presents interpretive ambiguities. The standard used for serological evaluation has been the serum virus neutralization test. Recently, with the introduction of gene deleted vaccines and their specific ELISA counterparts, vaccination of herds and differentiation of immune response due to vaccination rather than from contact with the wild PRV virus has been possible. Sensitivity of these ELISA tests, as well as the serum virus neutralization test approaches 100% when used 10 to 14 days post PRV challenge in pigs.[15] The specificity of these ELISA tests are not 100%[a] and it is assumed that either the initial tests were false positive or the individuals had undetectable antibody levels by the time of the whole herd test, two months after the initial statistical sampling.

Farm number 4 presents another particular point of concern. The initial statistical sampling identified one sow as being a PRV suspect, while none were determined to be PRV positive. On whole herd testing, however, four pigs were identified as seropositive and another 12 suspect. With a low prevalence (9.2%), if the suspect individual had been removed and the herd statistically retested, the farm would have again been at risk of being falsely classified as negative.

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[a]Personal communication from Quinton Tinnelli, IDEXX Laboratories, One IDEXX Drive, Westbrook, Maine

Finally, it should be reemphasized that the whole herd testing done in Carroll county was completed on the magnitude of a pilot project. These results warrant further testing, including herds found to be PRV seronegative on the final CCPP statistical sampling, to verify the findings and validate revision of the state of Iowa policies regarding the PRV eradication program.

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GENERAL SUMMARY

In order for the national pseudorabies eradication effort to proceed as quickly and economically as possible each state must cooperate with the program to the fullest extent of its ability. Approximately 25% of the nation's swine population is in Iowa, putting it in a unique situation. Iowa must strive to strike a balance between the regulations of the national program and the needs of its swine producers. The economy of Iowa is primarily agriculturally based. The implementation of a program having such a profound impact on the state's agricultural industry, as does the pseudorabies eradication program, must be carefully and fully considered.

Iowa has used the results of the eradication pilot project completed in Marshall county as a basis for establishing the random statistical sampling at a 95/20 level. Assuming the pilot project accurately represented the intraherd prevalence of the state's swine herds, this confidence and prevalence level is sufficient to meet the state's needs in the initial phases of the eradication program.

After following specific swine herd health management procedures, such as those of the Carroll County Pseudorabies Project, preliminary results indicate the swine producers of Iowa can expect to decrease their intraherd prevalence of pseudorabies. As the eradication program proceeds in Iowa the intraherd prevalence may decrease to such a level that, when testing at a 95/20 level, a false sense of accomplishment may develop due to not identifying as pseudorabies positive those herds with an intraherd prevalence below the 20% level. The swine industry and state officials are aware that, at some future time in the eradication

program, the testing guidelines will need to be adjusted to further the eradication process in a confident and scientifically sound manner.

Whole herd testing in Carroll county underscores the need for continued surveillance of the testing procedures as the eradication program advances. Funds must be encumbered to allow whole herd testing at time intervals, or at intervals of apparent prevalence, in order to continually validate the testing procedures needed to achieve pseudorabies eradication.