Studies on epidemiology and control of pseudorabies (Aujeszky's Disease) in Iowa swine

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TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF THE LITERATURE

Pseudorabies is an infectious disease found in many parts of the world. Outside the United States the disease is often referred to as Aujeszky's disease. Swine are generally accepted to be the reservoir host of the etiologic agent. Other animals may be affected incidentally and often fatally. These include most mammals and some birds species. A common name for pseudorabies is the "mad itch" which is descriptive of the clinical symptoms in farm animals other than swine. For example, cattle that have been licked on the mucous membranes or bitten by infected swine are known to develop intense pruritus at the site 3-5 days later. The head, flank, feet or perineal area are commonly affected sites. The animal often proceeds to violently lick, chew and rub the area to the point of self-mutilation. Hence, the name "mad itch". The bovine develops pyrexia and central nervous system derangement and invariably dies 6-48 hours after the onset of clinical signs.[6,18]

Etiology

Pseudorabies is caused by an agent in the Herpesviridae family of viruses. Most of the domestic animals are known to host at least one species of the Herpesviridae family. The following table lists some of the herpesvirus diseases

of the domestic animals.

Table 1. Common Herpesviral Diseases of the Domestic Animals

ANIMAL SPECIES	DISEASE
Bovine	Infectious Bovine Rhinotracheitis (IBR)
	Pustular Vulvo-vaginitis
Ovine	Ovine Pulmonary Adenomatosis
Canine	Hemorrhagic Puppy Disease
Feline	Feline Rhinotracheitis
Equine	Equine Rhinopneumonitis
	Equine Coital Exanthema
Porcine	Inclusion Body Rhinitis (Cytomegalovirus)
	Pseudorabies (PR)
Simian	Mild oral lesions (Simian B virus)
Avian	Various Diseases of chickens, turkeys, ducks, etc.

Human beings are hosts for a number of viruses in this ubiquitous family. Some of the diseases believed to be caused by these agents include chicken pox (varicella), infectious mononucleosis, shingles (zoster) and herpes simplex types I and II syndromes. The common oral canker experienced by almost every person at one time or another is caused by the herpes simplex type I virus. The extremely deadly simian B virus carried by certain monkeys belongs to the family Herpesviridae.[12]

One may get the impression that the viruses mentioned above are not entirely species specific. By species specific it is meant that the particular viruses rarely infect animals other than their natural hosts (see table 1). There are notable exceptions to this general rule among the

herpes viruses. Often the infection of a non-reservoir host species results in a fatal encephalitis in this aberrant host. The simian B virus infection of humans for example causes such a syndrome. Pseudorabies virus follows this pattern with probably more species of animals than any other herpes virus. Humans and the great apes seem to be refractory to pseudorabies fortunately.

Herpes viruses as a group are DNA containing viruses. They are 120-180 nm in size, enveloped, and have cubic symmetry.[7] They tend to be respiratory pathogens and some are implicated in neoplastic disease. Many herpesviruses, including pseudorabies, establish latent infections in their reservoir hosts. It is believed that these infections may persist for the life of the animal/human.

Clinical Signs

The clinical signs of pseudorabies in animals other than swine principally involve the nervous system. The route of exposure is thought to correlate with the clinical picture. Cattle exposed by bites from swine are thought to develop the "mad itch" syndrome. The pathogenesis has been described.[6,18] Other animals susceptible to pseudorabies may develop this syndrome as well. Infection of cattle by the respiratory route has been reported in recent years with increased frequency.[2] Such animals may show few specific

clinical signs before death occurs. Fever, anorexia and malaise may be noted but often the veterinary clinician is simply presented with sudden death losses. Sheep are very susceptible to pseudorabies. The modified live swine vaccine is extremely hazardous to exposed sheep.[8] Cats and dogs presumably become infected via the oral route. Clinically, these animals may begin scratching and/or pawing at their throats. As the disease progresses, the following symptoms may be noted: pyrexia, excessive salivation, anorexia, central nervous system abnormalities, generalized paresis to paralysis, and death ensuing within 48 hours after the onset of clinical signs. (Rabies presents a similar clinical picture and hence the development of the name pseudorabies. The root "pseudo" is derived from the Greek words "pseudes" and "pseudein" (meaning false and to deceive).)

Although there has been a few reports of cattle surviving pseudorabies[18], it is generally believed that all aberrant hosts of pseudorabies die. Swine are the only known reservoir hosts of the virus.

The clinical signs of the infection in swine can be quite variable. The strain of virus, age and immune status of the pig are some of the factors involved.[27] Gustafson[18] describes the clinical response as a continuum from subclinical to death. Clinical signs of pseudorabies, as well

as many other herpes virus infections, tend to be more severe in younger animals. The greatest morbidity and mortality occur in the suckling piglet. Nearly 100% mortality can be expected in susceptible piglets under 2 weeks of age. Piglets born to covalescent or vaccinated sows may be highly resistant depending on the level of passive immunity. Affected piglets are a disheartening sight to veterinarian and producer alike. The baby pigs manifest fever, dyspnea, anorexia, and depression, followed by ataxia, intermittent convulsions, running fits, coma, and death. The course of the infection is usually less than 48 hours once signs are apparent.[18] Two other rare symptoms have been described: pruritus over the irritated area of the brain, and uclerative lesions on the planum rostrale. The pruritus is the swine sequel to the "mad itch" of other animals. The snout lesions parallel the human herpes simplex canker.

The clinical course of experimental infection of pigs from weanling to market age is well-established.[18] Slight sneezing may be apparent beginning on day 2 after inoculation. Pyrexia and anorexia develop around day 3. There may be occasional coughing beginning at this time. Depression and tachypnea develop around the 4th day. Excessive salivation may be noted. Neurologic symptoms may develop in pigs 4-7 days after inoculation. When central nervous system

involvement becomes evident the infection is usually fatal. Ataxia, blindness, intermittent seizures, and running fits are seen before coma and death ensue. The seizures are quite unique. The animal may appear normal or have a slight ataxia. All of a sudden, the pig's ears are drawn back and the head and neck are extended upward. The eyes are open wide and the poor creature appears to be looking at something above. The animal begins to shake uncontrollably. There is a movement backward and the hindlimbs collapse. The neck remains extended as the pig falls to one side or the other. The animal may paddle for a time while laying on its side. Then, after only a minute or so, the pigs gets up and returns to its previous condition. This pattern may repeat itself at various intervals. Eventually, the animal weakens and becomes recumbent. Death usually occurs within 24 hours of the onset of these symptoms. Sometimes general irritability, restless wandering, and/or large purple patches on the pinnae of the ear are all that are noted before death. Mortality is greatest 5-7 days after dosing with the virus. If recovery occurs it usually begins on day 7-8. In the field, a wide range of clinical signs may present themselves. When a susceptible herd is exposed to pseudorabies there may be no symptoms noted. Often there is simply a transitory anorexia noted by the producer. The full gamut of clinical signs may be noted in a particular

herd; however, in Iowa it is apparent that the subclinical infection may be the rule rather than the exception. Strain differences may be factors involved in the pathogenicity of pseudorabies.

Another syndrome described by some is a pneumonic form of the disease. The majority of reports are from European authors.[32] The affected pigs are described as feeders in the 150 pounds to market weight range. The clinical signs are reported to vary from mild coughing to respiratory distress and death. Parallel reports from the United States are few. If the syndrome does exist in this country, its significance has been underestimated.

The infection in adult swine is often mild. The clinical signs associated with experimental infection are dependent on the challenge dose.[3] Death losses of adult swine are rare in the field. The problem of interest to many producers is the ability of pseudorabies infection to penetrate the gravid swine uterus. Reproductive disturbances have been reported with infection at all stages of pregnancy. If pseudorabies infects the pregnant animal during the first 30 days of gestation, the feti may be reabsorbed.[18] A depressed conception rate and altered interestrus intervals may be noted in these animals. If pseudorabies infection occurs during the second and occasionally in the third trimester of pregnancy, spontaneous abortion may occur. When

pregnant sows/gilts are exposed during the last trimester of gestation, a high rate of stillborn, macerated and weak pigs may occur. The liveborn piglets can be infected in utero. Other viral infections as well as Leptospirosis may be responsible for similar reproductive problems.[30]

The pathogenesis and immune response to pseudorabies infection in swine have been described.[6,18,19,51,52] Cell-mediated immunity (CMI) is thought to be very important in containing the infection in swine. Pseudorabies specific T-lymphocytes can be detected very early in the course of infection; even as early as 4 days after experimental challenge.[19,52] Specific humoral antibodies are reportedly detected from 14-22 days after challenge. Although CMI is very important, seroneutralizing antibody level seems to correlate with protection to experimental pseudorabies challenge.[36]

Sequelae to Pseudorabies Infection

There has been interest in the sequelae to pseudorabies infection in swine. A negative effect of pseudorabies on swine rate of gain has been noted.[18] After a herd pseudorabies outbreak, some farmers report a depressed rate of gain in weanling and early growing pigs. Some producers have also reported that these pigs seem more susceptible to other diseases. One study suggests that there may be synergism between pseudorabies infection and Pasteurella

multocida bronchopneumonia.[15] Gustafson notes that reproductive problems in adult female swine may continue several months after infection.[18] Presumably, bacterial metritis and/or endometritis are responsible in these cases. The economic affect of acute pseudorabies infection on the individual producer ranges from minor to devastating. If sequelae are indeed common, they must have some impact on the producer also. Very little is reported in the scientific literature on this topic.

The mechanism whereby sequelae to pseudorabies infection are induced, is unknown. The dichotomous and complex immune system may be involved. Since other members of the Herpesviridae are known to affect the immune system of their hosts, the similar concern about pseudorabies virus isn't surprising. Cytomegalovirus infections of humans and mice have been shown to non-specifically suppress both humoral and cell mediated immune responses.[13,21,34] Marek's disease virus of chickens, bovine herpesvirus I, and other herpes viruses have similar affects.[13,33] The exact mechanism of this suppression has not been demonstrated. Herpesviruses may infect and alter the function of certain lymphoid cells.[25]

There are a number of techniques which are used to assess cell mediated immunity. In vitro techniques are widely used; however, the experimenter must assume that

findings obtained through the method used will correlate with the in vivo immune situation.[52] One commonly used in vitro technique is called lymphocyte blastogenesis. Peripheral blood lymphocytes are cultured in the presence of mitogenic substances. Phytohemagglutinin (PHA), concanavalin A (CON-A), and pokeweed mitogen (PWM) are often used as mitogens in such experiments. The lymphocyte blastogenic response to these lectins is independent of past antigenic exposure; thus, the response is non-specific in reference to antigen. The intent is to measure lymphocytic ability to respond to hypothetical antigens. Suppressed responses may be seen in immunosuppressed subjects. Bovine Virus Diarrhea (BVD) infection, for example, is well-known for its ability to impair the immune system. A depressed non-specific lymphocyte blastogenic response has been reported in cattle shortly after BVD challenge.[39] A similar effect has been noted after infection by herpesviruses, e.g., cytomegaloviruses, Infectious Mononucleosis virus[28] and Infectious Bovine Rhinotracheitis (IBR) virus.[13] One experimenter observed that pseudorabies infection did not seem to affect non-specific responsiveness to PHA, CON-A, or PWM.[48] The results of our experiments reveal that pseudorabies infection does suppress responsiveness to these selected mitogens.

The humoral portion of the immune system may also be

affected by viral infections. For example, infection with human and murine cytomegaloviruses can involve a period of time in which the humoral immune response is non-specifically suppressed. This suppression is usually assessed by measuring antibody response to test antigens. A non-specific picture of the humoral immune system is desired; therefore, antigens that are unrelated to the virus in question are used. Multiple and varied antigen types are desirable. It is also important to utilize a "killed" antigen for this assessment. An antigen that replicates in the animal body may lead to paradoxical results. To illustrate this, consider administering a "live" antigen to an animal with deficient cellular immunity. The "live" antigen may freely multiply to an unusually high level before cellular mechanisms gain control. The antigenic load available for humoral immunity is thus increased. Theoretically, this may lead to an elevated antibody response. One would have expected a depressed response in an immunocompromised animal; thus, a paradox is observed.

There is little mention in the literature of the affects of pseudorabies infection on humoral responses to subsequent antigenic stimuli. An experiment in Taiwan suggests that pseudorabies infection interferes with Hog Cholera vaccination. The authors speculated that pseudorabies viral replication in the pigs' lymphoid tissues may

be responsible for the vaccination failure.[25] The vaccine administered in the study was a modified live preparation. A deficit in cell mediated immunity cannot be ruled out in this case. Our studies here at Iowa State University may be the first reported on the humoral immune response during and subsequent to pseudorabies infection.

Epidemiology of Swine Pseudorabies

Numerous authors have studied the prevalence of pseudorabies specific antibodies in the swine population.[24,37,46] About 14% of Iowas' market weight hogs and 34% of the breeding swine have such antibodies. The evidence indicates that PR prevalence in the United States has increased in the last decade.[46]

The primary mode of transmission among swine is thought to be via aerosolized virus. Acutely infected and convalescent swine excrete high levels of infectious virus in their nasal secretions. On exhalation, virus-containing droplets are propelled into the air. The droplets are potentially infective to swine in the immediate vicinity. Some of these contagious droplets undergo dehydration to form droplet nuclei. These nuclei have the potential to traverse greater distances. It is not clear how long these nuclei remain infectious or what distances they may travel.

Foot and mouth disease (FMD), an infection caused by a picornivirus, may spread many miles via droplet nuclei. It

is thought that one virus particle carried by the wind may spread the disease after traveling many miles. FMD is thought to have crossed the English channel to Great Britain by just this method.[41] At least one British author believes pseudorabies may have spread over several miles, similar to FMD virus.[17] The evidence at this time for such transport via droplet nuclei is inadequate. Firstly, a minimum dose is probably required to induce pseudorabies infection.[3] Also, the herpesviruses in general are not as durable as viruses of some other families, for example the Picornividae. It has been demonstrated that double fencing appears to stop pseudorabies transmission from swine to cattle and sheep.[44] The practice of segregating replacement breeding swine[42,44] to conserve valuable blood lines often hinges on the assumption that aerosol transmission only occurs during close swine contact. This cleanup method allows pseudorabies free pigs to be maintained close to but not in contact with infected animals. Such separation has been effectively carried out at distances of 50 yards or less.

Oral exposure and infection of swine may occur. Although reproducing pseudorabies experimentally in pigs is unpredictable using an oral route of exposure, transmission to the domestic carnivores commonly occurs by this route.[16] It is unclear how prevalent cutaneous exposure is because swine do not develop the "mad itch" as do the other species.

Various species of wildlife may play a role in pseudorabies spread. Animals that commonly frequent hog lots in Iowa include raccoons, opossum, skunks, rats, mice, domestic cats and dogs, starlings, and sparrows. These creatures are susceptible to pseudorabies at least experimentally. It is unclear whether starlings, sparrows, or opossum become infected in field situations. Their role in mechanical spread of pseudorabies is also unknown. Raccoons are probably a dead end host of pseudorabies.[56] It is conceivable that a raccoon incubating the infection could carry the virus to another farm. The same situation could occur with dogs, cats, skunks, mice, and rats. No wild animal other than feral swine have been documented to survive pseudorabies infection; therefore, if these animals do indeed transfer the virus from farm to farm they probably do so during a rather short incubation and clinical period (2-5 days).

Mechanical transmission of the agent from premises to premises can occur, but clear examples are rare.[42] Pseudorabies virus is rapidly inactivated by common disinfectants, ultraviolet light, drying, pH extremes, and by warm temperatures. The survivability of the virus in the environment and on various fomites has been reported.[4,10,14,54] At 25 degrees celsius, the virus probably survives less than a week under ideal environmental conditions. Such conditions rarely exist and the virus usually survives a maximum of a

few days outside both the pig and the laboratory. At lower temperatures, the virus can survive longer periods of time. At temperatures around 4 degrees celsius, the virus may survive about a month.

An epidemiologically important aspect of pseudorabies infection in swine is the tendency for convalescent animals to develop latent viral infections. Numerous authors have described pseudorabies latency in swine.[5,9,31,33,40,49] Convalescent swine are considered to be potential non-symptomatic carriers of the virus and are assumed to be so for life. Latency explains the infection of susceptible herds via the introduction of clinically normal swine. The latently infected pig is probably the major vector in the spread of pseudorabies from farm to farm.[35] In times of stress, virus may be excreted in the nasal secretions of such swine. Expression of virus after a period of latent infection is often termed recrudescence. Latent herpesvirus infections, including pseudorabies, are well-known for recrudescent as well as latent periods of viral expression. Detecting latently infected swine is of prime importance in pseudorabies control programs.

Pseudorabies Control

In the United States, federal regulations apply to Aujeszky's disease. In 1979, restrictions were adopted that

restrict movement of seropositive swine. The regulations require serological testing of all swine being moved interstate.[55] When the diagnosis of pseudorabies is made in a particular herd, the farm premises are quarantined. In Iowa, swine from pseudorabies quarantined farms may only be sold to slaughter or to state-certified quarantine stations. The diagnosis of pseudorabies, as one might expect, brings dilemma connotations to the producer far more encompassing than the medical impact on the herd. The producer who sells breeding stock must deal with the monetary loss resulting from selling his or her swine at market prices. The economic impact on farrow to finish operators may be slight; however, the quarantine concept may be distasteful to them. Perhaps it is like the teenager who is told he can't go to the dance until he cleans up his room. Although he wasn't planning on going to the dance, he dislikes being being told what to do.

Iowa regulations require that the diagnosis of pseudorabies be laboratory confirmed. The laboratory tests commonly used include direct fluorescent antibody examination of tissue, virus isolation, and antibody titration. The serum neutralization (SN) test has become the serological technique of choice for pseudorabies antibody titration. The enzyme-linked immunosorbent assay (ELISA) has recently become widely used as a screening test. Serum neutralization procedures have been described by Hill et al.[20] The

antibody titer is expressed as the reciprocal of the highest neutralizing dilution of serum. Dilutions are usually two fold, e.g., 1/2, 1/4, 1/8, etc.; therefore, antibody titers are reported as 2, 4, 8, etc. The SN method in general is very reproducable; it is the standard to which other techniques of serum antibody titration are compared.

In the midwest United States, it is generally assumed that animals with pseudorabies specific antibody titers of 4 or greater have been exposed to the virus. Such animals are suspected of being latently infected with pseudorabies virus. Generally, latently infected animals have been shown to manifest antibody titers of 4 or higher; however, one author has documented a situation where swine presumably harbored the virus for a period of time without detectable antibodies.[43]

Vaccination

Both modified-live virus (MLV) and killed virus vaccines are marketed in the United States. The evidence indicates that all are very effective in controlling the clinical signs of pseudorabies.[1] Mass vaccination is probably indicated and effective in acute pseudorabies outbreaks.[29] Ideally, pregnant swine are vaccinated prior to breeding and again shortly before farrowing; optimal protection is thus transferred to the newborn litter via colostrum. Vaccination of weanling swine on farms with vacci-

nated breeding animals may not be efficacious. The circulating immunoglobulin-G in these young pigs interferes with the induction of active immunity. Colostrum derived immunity decreases to undetectable levels at 2-4 months of age.[22] There is evidence that vaccination may still be ineffective for a period of time after antibody levels have decayed below detectable levels.[36]

The diagnosis of subclinical pseudorabies can be more complicated if vaccine is used. Antibody titers may be detectable for up to 11-13 months after MLV vaccination.[1] At the present time and with current vaccines, direct differentiation between field infection and vaccination antibodies cannot be made.[55] As a general rule, vaccination derived antibody titers are lower than true infection titers. Multiple and selective serum samplings from the herd in question may be helpful in assessing herd infection status. One author suggests that titers of 8 or higher in a herd indicate field infection.[23] A new vaccine is expected to be marketed in 1986. The vaccine is of the killed variety but is unusual in that it consists of partial instead of whole virions. Experiments with this type of vaccine are becoming more prevalent and it is usually referred to as a subunit vaccine.[26,45] The pseudorabies subunit vaccine has direct application to Aujeszky's disease control. Antibody populations induced by the subunit vaccine are slightly

different from those due to field infection. A modified ELISA procedure is able to directly differentiate between animals infected with virulent virus versus those vaccinated with this vaccine.[38] The subunit vaccine will offer the veterinarian another tool in the discernment of herd infection status.

The role of vaccine in the elimination of pseudorabies infection from a particular farm or area has been given little attention from an epidemiological viewpoint. It is known that vaccination does not prevent pseudorabies infection. There are also good indications that latent field strain infections occur concomitantly with vaccinated swine. Vaccinated as well as non-vaccinated swine derived from infected environments must be regarded as potential disseminating sources of Aujeszky's disease according to some experimenters.[11,31] There may be situations, however, where vaccination is advantageous. There is evidence that it may shorten the period of viral shedding after infection.[11] In herds where pseudorabies virus is "cycling" among hogs, the use of vaccine may slow the transmission cycle. In eliminating the infection from herds of swine, it is helpful if the transmission cycle is relatively quiescent. Vaccination is also highly recommended when using the offspring segregation method of herd cleanup. Vaccinated breeding gilts and sows convey passive immunity to the newborn piglets. The

immunoglobulins and lymphocytic cells are transferred almost exclusively through the ingestion of colostrum by the piglet. This immunity has been shown to protect suckling pigs from infection during their intimate association with the mother and their inhalation contact with other adult swine in the farrowing unit. This protection usually persists through the nursery and grower periods (units), i.e., for up to 3 months. If transmission to such swine occurs, it is usually in the finishing unit. Breaking the transmission cycle during the suckling period is critical to the success of the offspring segregation procedure.[4]

Herd clean up

In order to remove an existing quarantine, the producer must develop a serologically negative herd. Usually, this involves close veterinary consultation. Cooperation at all levels of management is very important in this endeavor. Other factors that must be considered include: the type of operation, isolation facility availability, farm layout, prevalency and original source of infection, availability of suitable replacement swine, neighborhood infection status, and producer determination.[42] A thorough epidemiologic evaluation is necessary. Economic considerations are paramount from the owner's viewpoint. Financial factors considered include: genetic value of stock, direct costs of

cleanup, indirect costs of cleanup, e.g., extra labor involved, and potential benefits of establishing a pseudorabies free herd. Many of the factors involved in eliminating Aujeszky's disease from herds of swine are described by Thawley, Gustafson, and Beran.[42]

Three basic methods of cleanup are described. An outline of these is found in table 2. Each method has advantages and disadvantages; the use of one method over another depends on the particular herd circumstances. In general it is best to attempt cleanup in the warm months of the year.

Table 2. Commonly Used Methods of Pseudorabies Elimination

1) Depopulation and Repopulation	
ADVANTAGES	:High rate of success
DISADVANTAGES	:High direct costs, loss of herd genetic character, producer down-time period required
2) Test and Removal	
ADVANTAGES	:Less costly than depopulation, retains herd genetic character, down-time usually not necessary.
DISADVANTAGES	:May not be effective in certain instances.
3) Offspring Segregation	
ADVANTAGES	:Least costly, retains herd genetic character, down-time not required.
DISADVANTAGES	:May require a longer cleanup period, may not be effective in herds with continuous virus cycling, requires isolation facilities.

Pseudorabies virus is more susceptible to inactivation in a warm environment. Colder weather may lead to additional stress, recrudescence, and viral transmission in swine housed outdoors or in environmentally uncontrolled units.

Depopulation and repopulation involves sending isolated infected groups or more commonly entire herds to slaughter. Progressive depopulation is recommended; breeding animals are removed as litters are weaned and growing pigs are sold to slaughter as they reach market weight.[42] After all swine have been removed, the premises are decontaminated. The buildings are cleaned with detergents and high pressure sprayer equipment. It is important to remove all organic material when cleaning; disinfects are much less effective in the presence of organic material. After cleaning, the surfaces are sprayed with a disinfectant and allowed to dry. The cleaning, disinfection, and drying should be repeated. Feeders should be emptied, cleaned and disinfected. Ventilation systems and other equipment should receive similar treatment. It is recommended that pits be pumped and cleaned at the same time. Outside lots may be scraped down to clean earth. Dry conditions are detrimental to viral survival and thus are preferred. The premises should sit idle for at least a 30 day period.

After the period of idle time, swine are again introduced to the premises. These swine should be from a herd

with a well-established pseudorabies-free record. Precautions should be taken to prevent re-infection. Barrier control of wildlife may be indicated. New herd additions should be tested before purchase and isolated upon arrival. It is recommended that these newly acquired swine be isolated for a period of 30 days and be tested again before release into the herd. The last precaution is critical because the carrier hog is the most common source of the infection. The long-term success of depopulation/repopulation as well as the other methods hinges on preventing re-infection.

Depopulation and repopulation of swine is a very effective method of ridding farms of Aujeszky's disease. If correctly performed, it assures that the herd will be free of infection at least for a short term period. It also allows the producer the potential to replace the herd with both genetically superior and disease free swine. Extensive pseudorabies testing and use of vaccine are not required. One disadvantage of depopulation and repopulation is the high direct cost. The procedure necessitates a time period where swine are absent; thus, the producer's cash flow is interrupted. The down-time period is usually the mostly costly aspect of this cleanup method. Depopulation is also not conducive to retaining genetically valuable swine.[42]

The test and removal procedure, as the name implies,

involves removing seropositive animals after testing. Usually, the entire breeding herd is tested. The seropositive animals are immediately removed from the herd and the testing is repeated in 30 days. Significant reduction in the seropositive rate should be noted after a few tests. Thawley et al. state that if greater than 1% of the breeding herd is seropositive after 4 tests, another strategy should be considered.[42] Test and removal is shown to be an effective procedure in certain instances.[55] It is recommended in herds where the seropositive rate is less than 50%, clinical signs are absent, and there is evidence that virus transmission is decreasing. It is not likely to be effective if the infection is active and spreading or if ventilation is such that all pig ages share a common air supply.

The advantages of test and removal over depopulation and repopulation include lower costs, allows for retaining genetic material, less disruption of management, and no producer down-time. Some of the disadvantages include it may not be effective in certain instances and extensive serological testing may be needed.

Offspring segregation is the third basic cleanup procedure. There are variations of this basic plan which have been described by Thawley et al.[42] Some sort of isolation facilities are necessary. Piglets are weaned as early as possible and selected females are placed in the isolation

facilities. These young gilts are tested periodically as they mature. If pseudorabies infection becomes evident, the swine are removed and the facilities are prepared for a new segregated group. When the group remains free of neutralizing pseudorabies antibodies, as is hoped, the gilts are prepared for breeding. Strict isolation procedures must be maintained at this point. The animals are bred with pseudorabies-free boars which also have been maintained in isolation. The original infected breeding herd is removed and usually sent to slaughter. The buildings housing the original herd are thoroughly cleaned and disinfected. The seronegative gilts are brought into the decontaminated facilities and proceed to farrow. Seronegative progeny of these animals gradually replace the swine in nursery, grower, and finisher facilities in the case of a farrow to finish operation.

The basis for the success of offspring segregation depends on lack of pseudorabies virus transmission between mother and her litter. A number of factors may be involved in this. There is evidence that the stress of farrowing may initiate virus shedding in latently infected sows. This recrudescence may be more of a concern in 1st litter gilts.[4] Also, colostral immunity in the piglets may convey some protection against latency producing infections, as described earlier. There is evidence, however, that if infec-

tion is active and spreading in the breeding herd, offspring segregation should be delayed or another cleanup strategy may be indicated.

Offspring segregation is compatible with retaining the genetic character of the herd. Very little producer down-time is required because the original infected breeding herd may be gradually depopulated. Some of the disadvantages include the need for isolation facilities, it may be ineffective in certain circumstances, and a longer time period may be needed to establish a seronegative herd.[42]

STATEMENT OF THE PROBLEM

This research will relate to pseudorabies epidemiology in the following areas:

1) The sequelae to pseudorabies infection.
   a. Is there experimental evidence that sequelae to Aujeszky's disease occur and what may be the mechanism of such?
   b. Does the infection affect the humoral or cell mediated immune system?

2) The control of swine pseudorabies in Iowa.
   a. Can Aujeszky's disease be eliminated from an endemic geographic area?
   b. What procedures are affective in controlling the infection and eliminating the disease?

If sequelae can be demonstrated, the impact of pseudorabies on the swine industry may be greater than currently believed. Also, the questions relating to pseudorabies control become more pertinent. The infection can be eliminated from individual farms. Pseudorabies has also been eliminated from various geographic areas with low incidence rates.[53] The following studies explore answers to these questions as well as provide further insight into pseudorabies epidemiology.

SECTION I. STUDIES OF SEQUELAE TO PSEUDORABIES INFECTION IN SWINE

Studies of Sequelae to Pseudorabies Infection in Swine

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SUMMARY

Three experiments were designed to evaluate humoral and cell mediated immune system parameters in pseudorabies (PR) convalescent pigs. Humeral responses to a modified-live virus transmissible gastroenteritis vaccine were significantly depressed in PR convalescent pigs versus PR-free control swine. Primary humoral responses to heat-killed Brucella abortus str. 19 were significantly higher in PR convalescent pigs. The brucella antigen was given 5 days after the inoculation of swine with PR virus. Primary humoral responses to ovine erythrocytes were similar in both PR convalescent and PR-free control swine. Lymphocyte blastogenic (LB) responses of swine peripheral blood lymphocytes to selected mitogens were also measured. During the period of time from 5 to 17 days after PR virus inoculation, LB responses to selected mitogens were significantly depressed in PR-infected animals. The responses returned to normal levels one month after PR virus inoculation. Results of these studies illustrate subtle changes in immune system parameters, especially during and shortly after active PR infection. Two other experiments attempted to demonstrate sequelae to PR infection. There was no evidence to indicate that previous pseudorabies infection exacerbated Salmonella choleraesuis var. kunzendorf nor Hemophilus pleuropneumonia infection.

INTRODUCTION

Pseudorabies (PR) can be an economically important disease to afflicted swine producers. An acute clinical outbreak may be devastating to a susceptible herd. High mortality is often experienced by swine in the early weeks of life. Pregnant animals may abort or farrow weak infected piglets. Losses may not be restricted to the clinical disease itself. Due to federal regulations, quarantines are imposed on infected herds.[9] Individual seed stock producers subject to quarantine are required to sell stock to slaughter only. The revenue lost to such producers can be substantial. Farmers that control the infection through vaccination must bear the costs of vaccine and additional labor.

Some Iowa producers have reported that acute pseudorabies outbreaks induce stunting in their growing pigs. Some farmers have expressed concern that these animals are also more susceptible to other swine diseases. There is very little in the scientific literature on this topic. Gustafson reported that pseudorabies convalescent swine may have a lower rate of gain after clinically apparent infections. He also reported that sows/gilts which have aborted may have a prolonged period of infertility.[4] A study at the University of Minnesota gave some evidence that pseudorabies convalescent pigs are more susceptible to <u>Pasteurella multocida</u> induced bronchopneumonia.[3] The frequency, extent,

and mechanism of such sequelae to pseudorabies infection are unknown. Taiwaneze experimenters have noted Hog Cholera vaccination failures in pseudorabies convalescent swine. The authors also speculated that the mechanism of such failures may be directly related to the affects of pseudorabies infection on the immune system.[6]

The purpose of the following studies have been:

1) To ascertain whether or not there is experimental evidence of sequelae to pseudorabies infection in swine and their possible relationship to field reports.

2) To assess the affects of pseudorabies infection on certain humoral and cell-mediated immune system functions and their implications.

MATERIALS AND METHODS

Basic Procedures

The serum neutralization procedures used in the following experiments have been described by Hill et al.[5] The lymphocyte blastogenesis procedure was performed according to Roth and Kaeberle.[8] The brucella microtiter agglutination techniques described by Brown et al.[1] were utilized in experiment 4. Ovine erythrocytes were obtained from a single adult wether and were used in agglutination tests as well as antigen preparations of experiments 3 and 4. Measurement of erythrocyte agglutinating antibody titers were made following procedures based on those of Osborn et al.[7] Data obtained through serological testing were evaluated with student's T tests and analysis of variance tests.

The analyses of lymphocyte blastogenic (LB) data were based on stimulation indices and corrected counts. The stimulation index is defined as [counts per minute (CPM) for treated cells]/[counts per minute for untreated cells]. A corrected count is defined as the difference in counts per minute of treated and untreated cells (CPM treated - CPM untreated). The analysis of variance procedure was applied to these data values. For the presentation of lymphocyte blastogenic data in graphic form, the values from groups were converted to a percentage of control value. This was accomplished by calculating a mean value for each parameter

of the control group each day. The value for each parameter of each individual animal in the PR-convalescent group was then calculated as a percentage of the mean control value for the corresponding day. The following equation was used: Corrected count/mean control CPM for the particular day.[8]

In order to facilitate the expression of time intervals involved in the following experiments, Day 0 is defined as the day of PR inoculation.

Experiment 1

Twenty-seven six-week-old swine were randomly assigned to 2 groups. The pigs were housed in separate isolation facilities. One group of 23 swine were challenged with 1000 plaque forming units (PFU) of pseudorabies virus (Iowa strain). The other group of 4 swine were maintained as pseudorabies free control animals. At 34 and 48 days after pseudorabies inoculation, TGE vaccine[1] was administered to each pig in both groups. Serum samples were obtained weekly for specific pseudorabies (PR) and transmissible gastroenteritis (TGE) viral antibody titration. Whole blood samples were collected for lymphocyte blastogenic assay.

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[1]Fort Dodge Laboratory, Fort Dodge, Iowa. Modified-live virus vaccine.

Experiment 2

Two groups of 8 swine each were established and housed in a manner similar to experiment 1. One group was infected with pseudorabies on day 0. The remaining animals were maintained as a negative control group. The pigs were 2 weeks old at the time of PR inoculation. On day 21, 5 animals in each group were challenged intratracheally with a standardized dose of Salmonella choleraesuis var. kunzendorf. Clinical responses after inoculation of salmonella were recorded for a 6 week period after inoculation. Rectal temperatures were noted daily.

Experiment 3

Two groups of 8 pigs each were established in a similar manner to the previous experiments. One group was infected with pseudorabies at 4 weeks of age (day 0) and the other was maintained free of the infection. All pigs in each group were inoculated with a ovine erythrocyte suspension on day 45. The suspension was prepared by washing sodium citrate treated whole blood 3 times with a buffered saline solution. The suspension was centrifuged and the fluid portion decanted. Saline was added to reconstitute a 50% suspension. All swine were injected in the neck musculature with 5 ml. of the preparation. Serum samples were collected weekly for erythrocyte agglutination testing. Whole blood

samples were also obtained for lymphocyte blastogenic assay. The following mitogens were used: Phytohemagglutinin (PHA), Concanavalin-A (CON-A), and Pokeweed mitogen (PWM).

Experiment 4

Twenty-four four-week-old swine were obtained from 3 different litters. Fifteen pigs were randomly assigned to one group and 9 to another. The 2 groups were housed separately. Each animal in the larger group was inoculated with 120 plaque forming units (PFU) of pseudorabies virus (Iowa strain). Clinical symptoms were recorded. Five days after PR virus inoculation, 2 antigen preparations were administered: Ovine erythrocytes and heat-killed Brucella abortus str. 19. The ovine erythrocyte suspension was prepared and given as in experiment 3. Brucella antigen was standardized to 4×10^{10} cells/dose. Both antigen preparations were given intramuscularly to each pig in both groups. Sera and whole blood samples were collected 2 or 3 times per week throughout the experiment. Sera were assessed for antibody titers to Brucella abortus str. 19, ovine erythrocytes and PR virus. Lymphocyte blastogenic (LB) responses to PHA, CON-A, and PWM were measured.

Experiment 5

The pigs from experiment 4 were utilized. The same facilities and group assignments were kept. Subgroups were

created in a random fashion:

Group	Number of pigs	Pseudorabies status	Treatment
A	3	free	none
B	6	free	dexamethasone
C	3	infected	none
D	11	infected	dexamethasone

Dexamethasone was injected intramuscularly 5 consecutive days at a dose of 1 mg/kg. The first treatment day was 34 days after the PR inoculation (day 34). Nasal and tonsil swabs were collected daily during steroid treatment and 1 week thereafter. Swabs were placed in 1 ml of saline-G for transport to the laboratory. The transport media was cleared of particulate matter by centrifugation. Davies et al. have described the culturing procedures used.[2] The presence of pseudorabies virus was indicated by the presence of typical cytopathic effect. Nine days after the last dexamethasone treatment (day 57), all pigs were infected with a dose of Hemophilus pleuropneumonia (serotype 5) equivalent to 5×10^6 colony forming units. The animals were inoculated intratracheally. The pigs were monitored clinically for 3 days. Rectal temperatures were recorded twice daily. Three days after inoculation the surviving animals were necropsied. Lungs were carefully examined and photographed. Necrotic areas of the lungs were outlined and a determination was made of the amount of lung parenchyma involved. The analysis of variance procedure was applied to the data values.

RESULTS

Experiment 1

Three pigs in the pseudorabies (PR) inoculated group survived the infection. Specific humoral and cell-mediated responses to PR antigen were noted in these animals 10 to 14 days after inoculation. Specific neutralizing antibody responses to TGE virus were noted four days after vaccination. The control group responded with significantly higher ($p < .001$) antibody levels than the PR convalescent group over the period of time after vaccination (days 34-84). The antibody responses to TGE vaccine are depicted in figure 1. The control group also produced higher stimulation indices to phytohemagglutinin (PHA) than the PR convalesent group, but the difference was not statistically significant.

Experiment 2

Serum neutralization results indicated that each animal in the pseudorabies convalescent group developed specific PR virus antibodies. Pigs in the control group remained free of such antibodies for the duration of the experiment. Following the inoculation of salmonella, the following clinical symptoms were noted in the first 2 weeks: pyrexia, diarrhea, depression, anorexia, and dehydration. All animals displayed these symptoms and some developed cutaneous ecchymotic hemorrhages as well. The clinical picture was

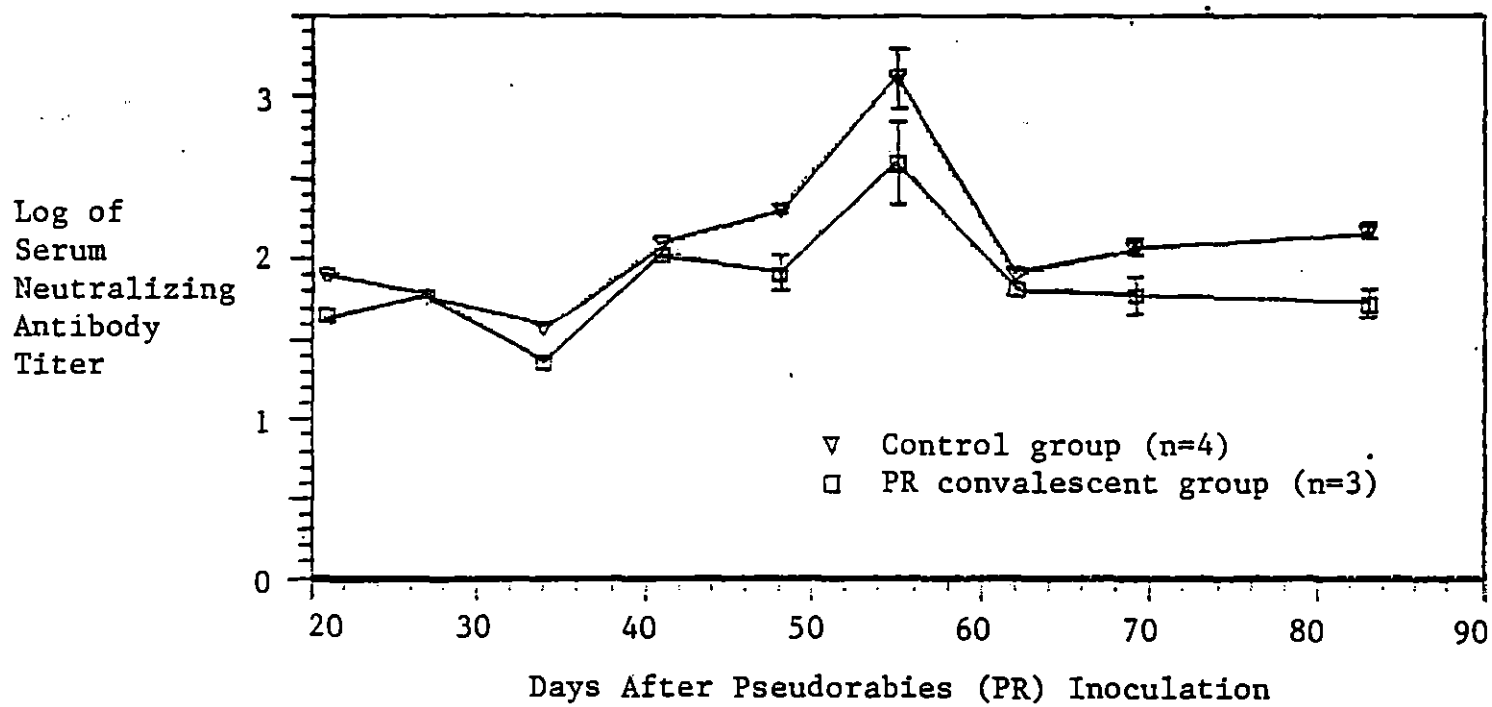

Figure 1. Mean Antibody Response of Pseudorabies (PR) Convalescent and PR Free Swine to a Modified-live Virus Transmissible Gastroenteritis (TGE) Vaccine Administered on Days 34 and 48

very similar in the 2 groups of pigs. Pyrexia and other clinical symptoms were also noted in uninoculated contact swine 24-48 hours after signs of disease appeared in the inoculated pigs. The symptoms indicated that there was rapid bacterial transmission to the contact animals. Two pigs in the control group died during the 2 weeks immediately after salmonella inoculation. During the next 4 weeks, the following symptoms were noted in some of the swine from each group: diarrhea, weight loss, and death loss. Three additional pigs from each group died during this time period. No difference in the severity of clinical salmonellosis was noted between the groups. Rectal temperature responses were likewise similar.

Experiment 3

All pigs in the infected group developed neutralizing antibodies to PR virus. Animals in the control group remained free of PR virus specific antibodies for the duration of the experiment. Pigs 6-7 weeks convalescent to pseudorabies infection expressed statistically similar LB responses to PHA, CON-A, and PWM as compared with control animals. Primary antibody responses to the ovine erythrocyte preparation were also similar in the 2 groups (see figure 2).

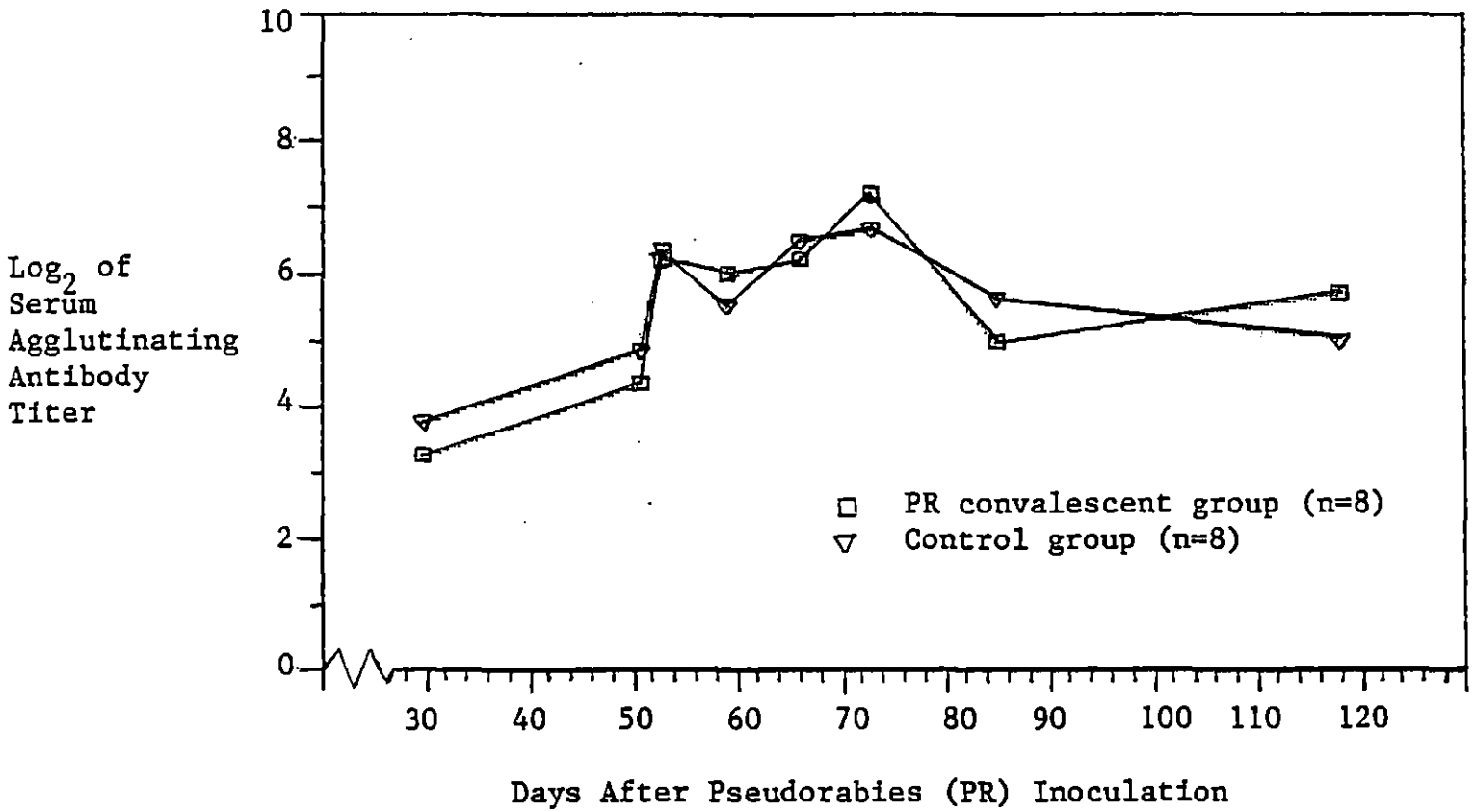

Figure 2. Mean Primary Antibody Response of Pseudorabies (PR) Convalescent and PR Free Swine to an Ovine Erythrocyte Preparation Given Intramuscularly on Day 45

Experiment 4

Two pigs developed central nervous system disturbances after PR virus inoculation and one of these pigs died. Three other animals in the PR convalescent group did not develop neutralizing antibodies to PR virus. It was concluded that these 3 pigs were not infected; therefore, data from them were not included in the analyses. Most of the animals became PR seropositive by day 23 after inoculation. The non-inoculated group of pigs remained free of PR virus antibodies for the duration of the experiment. The agglutinating antibody responses to ovine erythrocytes and Brucella abortus str. 19 are depicted in figures 3 and 4, respectively. The response to the brucella preparation was significantly higher ($p < 0.001$) in the PR convalescent group for the period from day 9 to 19. There appeared to be no significant differences between antibody responses to the ovine erythrocytes in the two groups.

Mean lymphocyte blastogenic (LB) responses are depicted in figure 5. In the time period from day 5-17, lower LB responses to PHA, CON-A, and PWM were noted in the PR convalescent group than in the control group. The differences in two groups' LB responses to CON-A and PWM were statistically significant ($p < 0.02$) for that period of time. Untreated lymphocytes from control pigs had significantly higher direct counts ($p < 0.01$) than the cells from PR

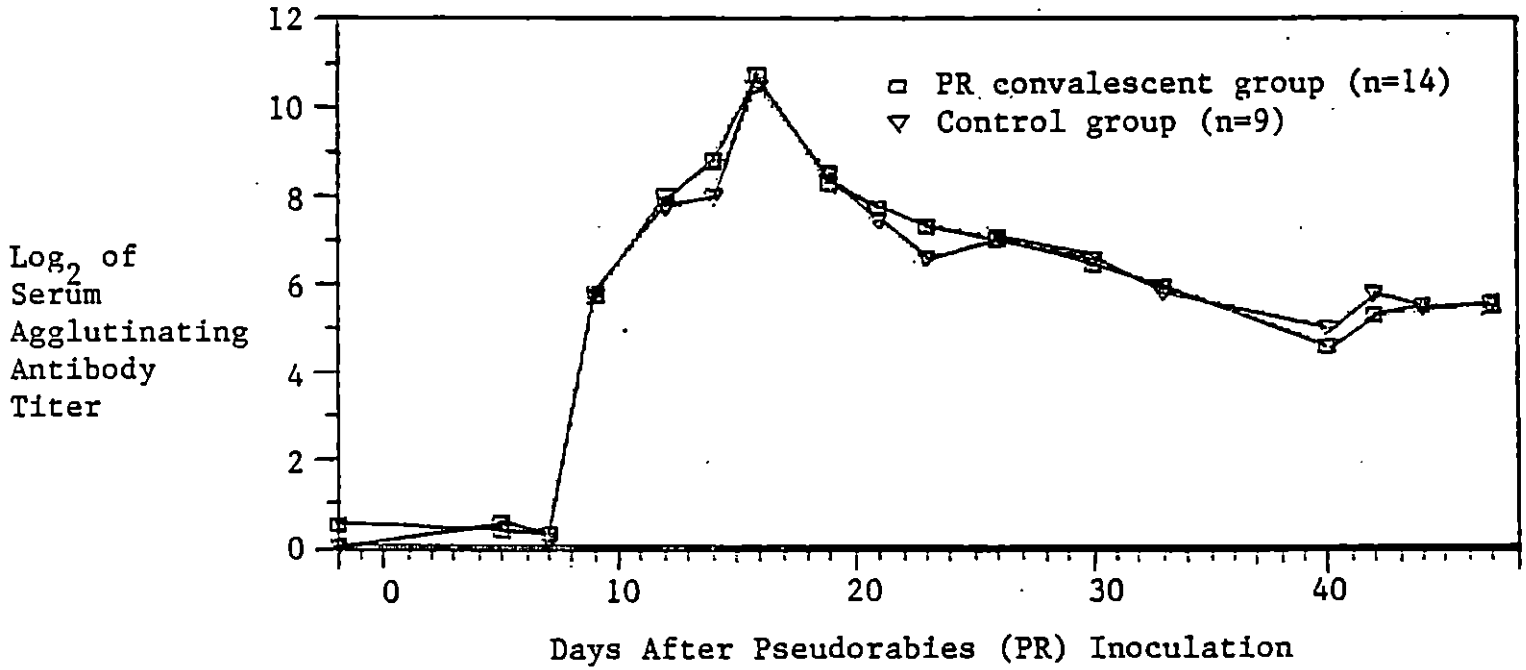

Figure 3. Mean Primary Antibody Response of Pseudorabies (PR) Convalescent and PR Free Swine to an Ovine Erythrocyte Preparation Given Intramuscularly on Day 5

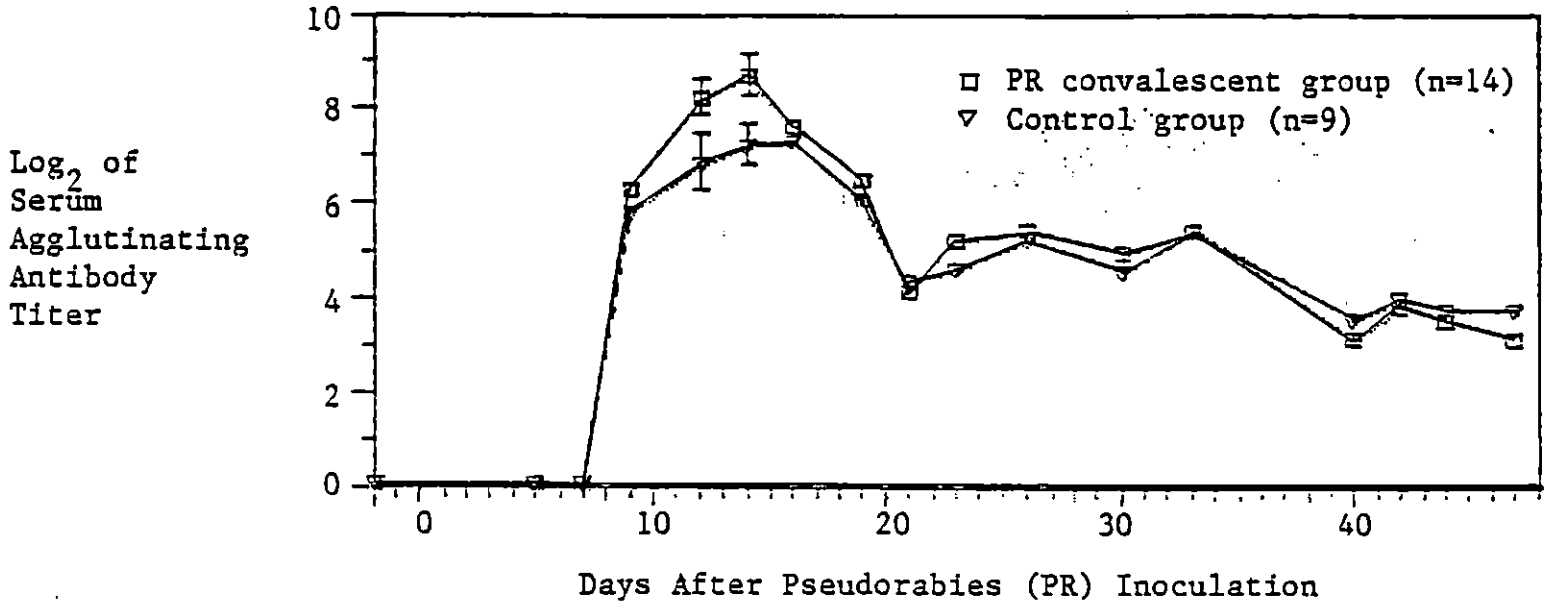

Figure 4. Mean Primary Antibody Response of Pseudorabies (PR) Convalescent and PR Free Control Swine to a Killed Preparation of Brucella abortus str. 19 Given Intramuscularly on Day 5

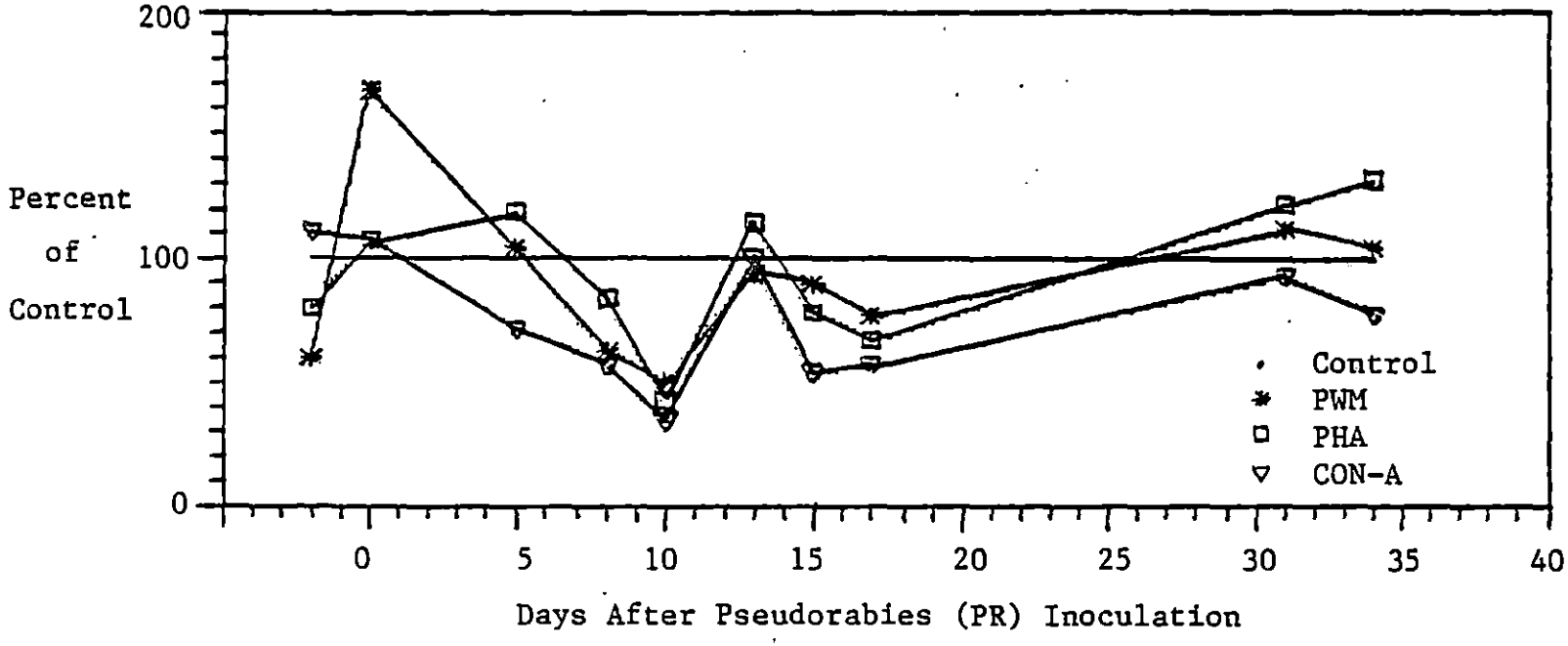

Figure 5. Mean Lymphocyte Blastogenic Responses of Pseudorabies Convalescent Swine (n=14) as a Percent of Un-infected Control Swine (n=9) for the Selected Mitogens Phytohemagglutinnin (PHA), Concanavalin-A (CON-A), and Pokeweed Mitogen (PWM)

convalescent pigs for the same period. Mean LB responses to these mitogens were statistically similar 30 and 34 days after PR challenge.

Experiment 5

Three pigs did not seroconvert to pseudorabies and data from these animals are not reported here. Of the remaining 11 animals, 5 shed pseudorabies virus on 2 or more days during the collecting period. Pigs 246, 248, and 392 died before the scheduled necropsy time and were examined shortly after death. The clinical symptoms of hemophilosis were equally severe between the groups. Tachypnea, pyrexia and anorexia were noted in most of the swine. Group average temperature responses were nearly identical. The virus isolation and postmortem data are given in table 1. The litter of origin of the randomly assigned pigs had a significant affect on the extent of necrotic lesions ($p < 0.05$). Both dexamethasone treatment and pseudorabies infection status affects were insignificant. There is no evidence to suggest that previous pseudorabies infection had any affect on the necrotic lesions of acute hemophilosis.

Table 1. Swab test results and extent of acute necrohemorrhagic lesions at necropsy

Group	Pig #	Litter #	Virus isolation	% lung involvement	
A	248	3	NA[a]	100	
	366	2	NA	5	$\bar{X}$ = 33
	393	1	NA	0	
B	248	3	NA	0	
	363	2	NA	10	
	364	1	NA	48	
	365	1	NA	0	$\bar{X}$ = 11
	386	3	NA	0	
	389	2	NA	5	
C	369	1	-	0	
	390	2	-	0	$\bar{X}$ = 0
D	246	3	+	50	
	362	2	-	18	
	368	1	-	0	
	370	3	+	0	
	387	3	-	43	$\bar{X}$ = 19
	388	1	-	0	
	392	2	+	42	
	394	1	+	20	
	395	1	+	0	

[a]NA Not Applicable, - negative, + positive virus isolation culture during 2 weeks preceding Hemophilus inoculation.

DISCUSSION

Demonstrating synergism among infectious disease agents is a difficult task. Many factors are probably involved. The nature of the agents and immune status of the host may contribute. The length of time between infection with one agent and the other is also important.

A deficit in humoral or cell-mediated immune system parameters may give credence to the idea that pseudorabies convalescent pigs are more susceptible to other subsequent infections. The results of experiment 1 indicated that PR infection affected the humoral response to a modified-live transmissible gastroenteritis virus vaccine. Pseudorabies convalescent swine responded with significantly lower neutralizing antibody titers as compared with control pigs. More research is needed to determine the exact mechanisms involved, including interferon induced viral interference and possible direct PR virus action on certain lymphocyte populations.

Experiments 3 and 4 attempted to assess the humoral responses of PR convalescent pigs to killed antigens. In experiment 3, an ovine erythrocyte preparation was given to pigs 6-7 weeks convalescent to PR infection. Similar primary responses was observed in these pigs versus those from PR-free control swine. Two different antigen preparations were administered 5 days after PR inoculation in experiment

4. While primary humoral responses to ovine erythrocytes were similar, the responses of PR infected swine to brucella were different from those of PR-free control swine (figures 3 and 4). It is not clear how or why PR infected pigs developed higher antibody titers to heat-killed Brucella abortus str. 19. Certain populations of lymphoid cells are probably either directly or indirectly affected by pseudorabies infection.

Lymphocyte blastogenic (LB) assay of swine peripheral blood in experiments 1, 3 and 4 reveal some general patterns. In the first few days to 2 or 3 weeks after PR infection, swine appeared to display depressed peripheral blood LB responses to PHA, CON-A, and PWM. At 1 month convalescent to PR infection, LB responses returned to normal levels. These results contrast those presented by Van Oirschot et al. which do not suggest that LB responses are depressed in PR convalescent swine peripheral blood.[10] The experimenters' results were based on group sizes of 4 animals each. Our groups of 9 and 11 swine may have enabled us to more easily distinguish subtle differences in LB responses.

The results of experiment 2 indicated that the clinical salmonellosis experienced by PR convalescent pigs was indistinguishable from that of PR free pigs. Both acute and chronic salmonellosis syndromes were very similar in the two

groups. The PR infected pigs in experiment 2 were approximately 3 weeks convalescent to pseudorabies infection when dosed with Salmonella choleraesuis var. kunzendorf. Experiment 5 attempted to demonstrate sequelae shortly after the recrudescence of PR virus. A dexamethasone treatment schedule was used. The results of tonsil and nasal swabs obtained indicated that some of the pigs were shedding virus before dexamethasone and some continued to shed during the steroid treatment period. One pig showed a virus isolation pattern compatible with a recrudescence. Perhaps a higher dosage regimen would have been necessary to invoke virus expression in latently infected pigs. Virus was detected from 5 pigs on at least 2 different days during the 2 week period immediately preceding Hemophilus pleuropneumonia inoculation. Although the swine in the PR infected group were inoculated 6 weeks before subsequent hemophilosis, at least 5 expressed an active PR infection at that time. One might expect that at least these 5 animals might have been more susceptible to a subsequent hemophilus infection. The results of experiment 5 gave no indication however, that acute hemophilosis was exacerbated by previous PR infection.

The time period between pseudorabies infection and subsequent infections is probably a factor in the development of sequelae. Based on immunologic assessment of PR convalescent pigs in experiments 1, 3, and 4 there seems to

be evidence that the immune system is altered, at least subtly. The evidence suggests that this alteration is evident for a short period of time after PR infection. The period of time is probably less than 1 month. Manifestation of sequelae to PR infection may depend on their development during this short period of time.

Salmonellosis and hemophilosis after PR infection were described in experiments 2 and 5 respectively. These 2 studies were designed to demonstrate sequelae to PR infection under controlled conditions. There is no evidence to suggest that PR convalescent swine were more severely affected as compared with PR free pigs; however, one cannot conclude that sequelae to pseudorabies do not occur based on these results alone. Studies with other infectious agents and/or different circumstances may yield different findings. Indications are that such studies should center their attention to the first few weeks after pseudorabies infection.

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SECTION II. THE EPIDEMIOLOGY AND CONTROL OF PSEUDORABIES IN A MARSHALL COUNTY IOWA PILOT ELIMINATION PROJECT

The Epidemiology and Control of Pseudorabies in a Marshall County Iowa Pilot Elimination Project

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INTRODUCTION

Pseudorabies (PR, Aujeszky's Disease) is an infectious disease of domestic animals. Swine are the reservoir host of the etiologic virus, which is a member of the herpesviridae family. The agent is able to infect many other species of domestic mammals as well as wildlife. In all cases, the infection can be regarded as fatal in these aberrant host species. The name "pseudorabies" was coined by researchers in the early part of this century.[10] Like rabies, PR infected aberrant hosts display central nervous system derangement and invariably die after a short clinical course. Cattle especially, may experience a cutaneous form of the infection. This syndrome is referred to as "mad itch" because pruritus is extremely intense at the point of exposure. Aberrant hosts of PR infection develop severe encephalitis. Paralysis and death usually occur within 48 hours of the onset of clinical signs.[2]

The clinical manifestations of pseudorabies infection in swine may vary from inapparent to severe.[5] Suckling-age piglets are extremely susceptible to PR. Mortality in non-immune swine under 3 weeks of age may approach 100%. Clinical symptoms of PR in young pigs include anorexia, depression, pyrexia, convulsions and death. In older hogs, the infection is usually less dramatic. Transient anorexia and fever may be observed. Occasionally, older swine will

show central nervous system or pneumonic involvement. Pregnant swine may abort or reabsorb developing feti. Convalescent animals become latently infected with PR virus. During times of stress the agent may recrudesce in such pigs.[1,3,9,15] These swine probably harbor the virus for the rest of their lives.

Modified live virus and killed virus vaccines are available for PR. They appear to be very effective in preventing clinical symptoms. The administration of vaccine to herds undergoing clinical outbreaks is indicated and practiced.[8] Current serological tests are unable to directly distinguish the antibodies produced by PR vaccination from those of field infection. It has been noted that vaccine induced neutralizing (SN) antibody titers remain below 32.[17] Pseudorabies vaccinated herds with SN antibody titers greater than 16 are potentially infected with the field virus.

The long term affects of PR infection in herds of swine are difficult to measure. Some Iowa farmers have reported poor growth rates after PR outbreaks. An increased susceptibility to other infections has also been reported.

National interest in pseudorabies has increased during the last 2 decades. The United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS) coordinated serological surveys of slaughter swine

four times since 1974. Specific serum neutralizing antibody prevalence increased from 0.56 % in 1974 to 8.78 % in 1984 for the United States as a whole.[12] Federal regulations were adopted in early 1979 to "control and stop the escalating spread of pseudorabies".[13]

In 1983, the National Pork Producer's Council (NPPC) proposed to APHIS that pilot PR area control projects be conducted in several states. Financial responsibilities were to be shared by NPPC and APHIS. The objectives of these pilot projects are described in table 1. Five states were proposed and selected as sites for the projects. North Carolina, Pennsylvania and Wisconsin incorporated the projects into existing state PR control and elimination programs. A higher prevalence of PR antibodies were noted in Iowa and Illinois (see table 2). Illinois proposed Pike and Macoupin counties for the project study area. Iowa selected Marshall county.

The goal of the Iowa pseudorabies pilot project has been to test the feasibility of controlling and/or eliminating pseudorabies in herds in an endemic PR area. The Iowa project was based on the voluntary participation of Marshall county swine producers. Methods of cleanup were to be designed to minimize both changes in normal management procedures and interruptions in farm cash flow. The Marshall county pseudorabies pilot elimination project (MCPPEP) was

Table 1. Purposes of Pseudorabies Pilot Projects
(National Pork Producers Council)

A. Pilot projects should be designed to determine the practicality of area eradication of pseudorabies.
B. Pilot projects should be designed to provide definite answers as to whether pseudorabies eradication is achievable.
C. Government and producers must accept the results of the projects and modify approaches as necessary.
D. A technical advisory committee should decide technical aspects of pilot project designs.

Table 2. Pseudorabies Serological Surveys[12] on Slaughter Swine

Percent Positive by Serum Neutralization Tests					
Year:	1974	1977-78	1980-81	1983-84 Breeders	1983-84 Market 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

initiated in July 1983.

The purpose of this study is to report on the methods and results of the ongoing MCPPEP.

MATERIALS AND METHODS

Participation

The voluntary participation of the Marshall county pork producers was the basis of the Iowa project. Serum samples were to be collected on individual farm premises as opposed to slaughter plants; cooperation was vital. The Marshall county pork producer's association as well as the Iowa state veterinarian's office were instrumental in coordinating farmer participation. Practicing area veterinarians were contacted and encouraged to participate. Meetings of the county pork producer association were organized. The state veterinarian and others involved in the MCPPEP disseminated initial information at such meetings. There were opportunities for producers to both express opinions and participate in project decisions.

Another feature that encouraged participation was the attitude toward the use of formal quarantine. In the MCPPEP, formal quarantines were reserved for herds with clinical PR. They were not imposed on farms that were classified as infected based on serological test results. Instead, the risks of selling seropositive swine were carefully discussed with such producers. Verbal agreements to sell pigs to slaughter only were obtained from the operators. It was believed that the use of a formal quarantine system would discourage participation in the project.

Record Keeping

In the summer of 1984, a computer record keeping system was initiated. The purpose of the system was to simplify and speed the record keeping process. A sample of the report forms used is given in figure 1. The reports could be updated easily and quickly distributed to field veterinarians and individual producers.

Pseudorabies Diagnosis

The diagnosis of PR in project herds was usually based on results of serum neutralization (SN) tests. Serum samples were collected on individual farm premises. Practicing area veterinarians were contracted to collect and submit serum samples. A statistical sampling system was chosen in contrast to complete herd testing. Breeding stock producers, however, continued to test all animals as is required for maintaining PR-free status. Initial statistical samplings were based on a 95% probability of detecting 10% or higher infection prevalence. The recommended sampling schedule[16] was as follows:

Less than 100 hogs	test 26
100-200 hogs	test 27
200-1000 hogs	test 28
Greater than 1000	test 29

In most herds, a sample of both the breeding stock and the progeny were obtained. After initial sampling, a periodic schedule for monitoring infection was adopted by all farms.

Owner: FARMER, JOHN Address: MARSHALLTOWN

Herd No.: 64-01-18-LC Veterinary Practitioner: DR. HOGG

Location: MARSHALL COUNTY, IOWA TOWNSHIP, SECTION 05

Type of Operation: FARROW TO FINISH

I. Herd Sampling:

Test Date	Pig Age	# on Farm	# In Sample	Neg	Pos	Mean Titer	1:4	1:8	1:16	1:32	1:64	1:128	=>1:256
07/21/83	A	40	11	3	8	4.5	1	2	4	0	0	0	0
07/21/83	3MO		20	12	8	1.7	8	0	0	0	0	0	0
04/13/84	A		14	7	7	4.2	1	1	0	1	2	1	0
04/13/84	5MO		17	17	0	0	0	0	0	0	0	0	0
10/05/84	5-6MO		24	24	0	0	0	0	0	0	0	0	0
05/30/85	6 MOG		5	5	0	0	0	0	0	0	0	0	0
05/30/85	1YVS		20	19	1	0	1	0	0	0	0	0	0

II. Vaccine Used Before Entry Into Project: MLV (MLV or Killed)

III. Herd Plan: 2A Options: 1, 1A, 2, 2A, 3A, 3B, 3C

IV. Vaccine Used After Entry Into Project: Killed (Killed or Subunit)

Date	Number	Age	Date	Number	Age
11/--/83	34	P	06/06/84	41	A
11/28/84	43	A	06/14/85	41	A

V. Herd Cleanup Action

Month Began	Month Completed	Disinfected Units

A. Adults

Removal of Positive Breeding Stock

Rotation of Entire Breeding Herd JUN84 JUL84

Depopulation/Repopulation of Herd

B. Progeny

Segregation on Farm (Distances: 50 ft)

Segregation on Separate Premises

VI. Replacement Stock Purchased

Date Purchased Number/Sex Dates Tested

Risk Status: Low

Notes: Clinical, Reproductive, Chronic Disease. Changed from 3C to 2A on 11/84

February 23, 1986

Figure 1. Sample of the Farm Report Form Used in the Marshall County Pseudorabies Pilot Elimination Project

These additional samplings were limited to breeding stock and specific portions of the herd depending on individual herd circumstances.

Farm Classification and Pseudorabies Control

Herds were classified into one of 3 general areas based on initial sample results: PR-infected, suspect, and PR-free. If SN antibody titers were detected in any animals with no history of previous PR vaccination, the herd was placed in the infected category. If SN results showed animals with antibody titers of 32 or higher, generally the herd was placed in the infected category whether or not vaccination was being practiced. Herds, using PR vaccine were placed in a suspect category if maximum antibody titers of 16 were detected. On certain occasions, herds were placed in the infected category based on laboratory isolation of PR virus or clinical symptoms of pseudorabies.

Farms classified as PR-free were then subclassified based on the perceived risk of infection. The high risk category herds were determined based on the following criteria:

a. Herds within 1 mile of a known infected herd.

b. Herds on the perimeter or within the project that have contact with herds of unknown status.

c. Multiple premises herds where where one or more of the premises is outside the area or has contact with infected herds or herds of unknown status.

d. Herds with feeder pigs purchased from infected herds or from herds of unknown status.

All other PR-free herds were designated as low risk.

After individual herd epidemiologic assessments, each producer was presented with the results. The farmer's personal veterinarian as well as the project veterinarian were key people in making personal contact with producers. In infected herds especially, veterinarians from Iowa State University and APHIS were involved in herd classification. In such cases, a visit was arranged with the producer. Various cleanup strategies were discussed and a working plan was agreed upon. The producer, his veterinarian, and the project personnel received the written PR elimination plans shortly thereafter. One of the written cleanup plans is given in figure 2. In the cases where herds were designated free of PR, producers were consulted and a plan for monitoring and preventing infection was adopted.

Seven basic PR control plans were utilized for all project farms. The plans and a description of each are given in table 3. Each basic plan was a unique schedule of periodic testing and stressed either prevention or elimination of PR infection. Plans 3A, 3B, and 3C deserve special attention. Herds identified with these plans were thought to be infected with PR. The categories 3A, 3B, and 3C correspond to different methods of cleanup described by Thawley et al.[11] Depopulation/repopulation, test-and-removal, and

VII. Herd Plan Description Herd no. 64-10-11-JH

The breeding stock are maintained in 3 separate herds, one on the home premises and 2 on a separate premises. In September, 1983, pseudorabies virus was isolated from progeny of the home premises sows and the sows and progeny of this herd are seropositive. The sows and progeny of the other 2 herds are seronegative. All farrowing is on the home premises in the same farrowing house but on different schedules. Progeny of the 3 herds are maintained in separate pens in the same building but not isolated. Vaccination of breeding stock was inititated following recognition of infection and is currently performed twice yearly.

The infected sow herd should be rotated and as rapidly as possible, and all replacement gilts selected from the other two herds. Isolated all progeny at weaning and maintain them segregated from the breeding stock. Select all replacement gilts at 5.5-6 months. If all are negative, the entire vaccinated sow herd should be sold to slaughter and the units cleaned and disinfected. The gilts may then be moved into the gestation units and vaccinated. If any of the replacement gilts are seropositive, they should be sold to slaughter and the remaining stock tested in 60 days, or the entire replacement group may be sold and the segregation procedure started over. Replacement boars should be isolated and tested twice at 60 day intervals.

The herd should continue to be monitored by testing 25-28 progeny again at 3

months. If all tested animals are again negative, and when an all negative breeding herd has been established, the herd owner may enter plans 1 or 2. As long as there is evidence of infection in the herd, it must remain in plan 3C.

VIII. Change in Herd Plan: Date _____ Change to _____
Reason _____
(if new herd plan description, see next sheet)

IX. Date pseudorabies free herd established _____

X. Additional recommendations or comments

Figure 2. Example of written procedures for the elimination of pseudorabies infection which were supplied to the particular farmer and other individuals involved in the Marshall county pseudorabies pilot elimination project

Table 3. Basic herd plan classifications in the Marshall County Pseudorabies Pilot Elimination Project

Plan	Infection Status	Infection Risk Status	Serological Sampling Schedule	Killed Vaccine Usage	Comments
1	PRV free	High	3 months	No	Qualified negative herds
	PRV free	Low	3 months	No	
1A	PRV free	High	6 months	No	Modified qualified negative herds
	PRV free	Low	9 months	No	
2	PRV free	High	3 months	Yes	Control vaccinated herds
	PRV free	Low	3 months	Yes	
2A	PRV free	High	6 months	Yes	Modified control vaccinated herds
	PRV free	Low	9 months	Yes	
3A	Infected	-	3 months	Permitted	Depopulation/Repop.
3B	Infected	-	3 months	Permitted	Test and Removal
3C	Infected	-	3 months	Permitted	Offspring segregation

offspring segregation are commonly used PR elimination methods. Descriptions of each and their uses in the MCPPEP are appropriate at this time.

Depopulation/repopulation

Depopulation/repopulation is a highly effective method of eliminating PR from herds of swine. If correctly performed, it assures the elimination of the infection. The procedure involves sending all animals on the farm premises to slaughter. The swine housing facilities are then thoroughly cleaned and disinfected. The units are allowed to dry before cleaning and disinfection are repeated. A period of time is recommended before the reintroduction of swine; this period should be more than 30 days. Depopulation/repopulation can be an expensive cleanup method due to the need for operator downtime.

Project plan 3A corresponded to depopulation/repopulation. It must be emphasized that in Marshall county this category generally applied to feeder pig finishing operations, e.g., those farms that fed batches of swine for market. Operators that stopped production for whatever reason were also placed in this category. This plan, or any other for that matter, was not intended to put financial burden on producers. It was hoped that PR could be eliminated from herds of swine without financial hardship to the farmers. The use of depopulation/repopulation was thought to be

inconsistent with these policies and was intended only as a method of last resort.

Test-and-removal

Test-and-removal has been utilized successfully in eliminating PR seropositive swine from farms.[17] This clean-up method involves selectively removing seropositive animals from the herd. It is not likely to be effective in herds where PR viral transmission is evident. Extensive serological testing may be necessary. Also, this method is not recommended if PR sero-prevalence is high in the herd. Although not as effective as depopulation/repopulation, test-and-removal presents certain advantages, e.g., less costly, no downtime, retains inherent genetic material, and minimal management changes are necessary.

Project plan 3B involved a test-and-removal method of PR elimination. This strategy was reserved for those farms with relatively low PR prevalence. Test-and-removal procedures were thought to be inappropriate for most of the Marshall county infected herds due to the discovery that the majority (28 of 40) had greater than 60% PR sero-prevalence.

Offspring segregation

Offspring segregation (OS) has been a highly successful method of eliminating PR from herds of swine. Piglets are weaned as early as possible, preferably by 4 weeks of age.

Replacement gilts are selected and removed from the normal production system. They are placed in clean, disinfected isolation facilities (units). At periodic times, samples of these gilts are tested for PR specific SN antibodies. All of the animals in the group are tested at the end of a segregation period. If infection is evident, the facilities are emptied, cleaned, disinfected, and the process repeated. When enough seronegative bred gilts are obtained, the entire infected breeding herd is sent to slaughter. The farrowing facilities are cleaned and disinfected before the new seronegative breeding herd is brought in. The swine raising facilities are progressively emptied, e.g., nursery, grower and finisher unit(s). Cleaning, disinfection and drying of these facilities should be complete before progeny from the new seronegative breeding herd enter the system. A 30 day progressive downtime approach is recommended.

A breeding stock vaccination program may be utilized in an attempt to minimize viral transmission in the farrowing facilities. Offspring segregation offers lower costs, minimizes downtime, and retention of original genetic material as compared with depopulation/repopulation.

Project plan 3C corresponded to offspring segregation methods. Isolated facilities were needed to house a new replacement breeding herd. The facilities were varying distances from other swine. The longer the distance, the

more desirable the facility was. Some isolation units were as close as 20 yards from other pigs. Others were located on entirely different farmsteads.

Killed virus PR vaccine was used in project OS procedures.[1] The killed product was preferred over live virus preparations for the following reasons: 1) It was felt that the killed vaccine would be safer from the standpoint of its potential reversion to virulent virus 2) The killed vaccine could be given to pregnant animals with minimal concern about vaccine inducted abortions 3) The killed virus vaccine induces lower SN antibody levels which interfere less in the interpretation of serologically data. Usually, only the breeding female animals were vaccinated. On some farms there was imminent risk of clinical PR. In these herds, vaccination of progeny was deemed necessary.

It was recommended that producers vaccinate sow prior to breeding and again 3-4 weeks before farrowing. Replacement gilts were to be vaccinated at 5-6 months of age, again before breeding, and 3-4 weeks before farrowing. The basis of OS is preventing transmission of PR virus to the suckling piglets. From this perspective, it was believed that such a vaccination schedule would be helpful. By boosting the immunity of breeding stock we attempt to minimize recrudes-

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[1]Norden Laboratories, Lincoln, Nebraska.

cent viral episodes and thus viral transmission in the farrowing facilities. Also, maximal passive immunity is transferred via colostrum to the newborn pigs. This immunity may increase the viral threshold required to induce latent PR infections in these piglets.

The success of all herd cleanup procedures depend on locating the original source of infection and eliminating it. The most common source of PR has been the latently infected pig. Strict procedures for the isolation and PR testing of purchased swine is recommended. In the MCPPEP, it was recommended that new stock be isolated and tested upon arrival, not be released into the herd for a period of 30-60 days, and be tested before such release.

Criteria for Attaining Pseudorabies-free Status

Once cleanup procedures were underway, certain criteria had to be met in order to move a herd from an infected classification to a PR free one. Firstly, it was required that all infected breeding animals be removed from the premises. Additional criteria were required depending on the cleanup approach chosen. If offspring segregation procedures were used, two or more subsequent progeny samplings must give no indication of PR infection. If test-and-removal or depopulation/repopulation procedures were used, the entire breeding herd must be tested 30 days or more after removing the seropositive animals. The results of such tests must give

no indication of PR infection. When these criteria were met the herds could be classified as PR-free at the discretion of the state veterinarian.

Calculations and Descriptions

The results of serum virus neutralization tests are expressed in terms of the following parameters:

1) The PR-specific antibody titer[6] is the reciprocal of the final serum dilution which inhibits the viral infection of a test cell system. Dilutions are usually two-fold; thus possible values are 4, 8, 16, 32, 64, 128, and 256.

2) Initial (PR) prevalence

$$= \frac{\text{Number of Seropositive Animals}}{\text{Total Number of Animals in Sample (n)}}$$

3) The highest serum neutralizing (SN) antibody titer is the maximum PR-specific antibody titer in the sample of swine tested.

4) The geometric mean antibody titer is the average PR-specific antibody titer of the given sample. It is calculated as:

$$\text{Antilog}\left(\frac{\sum_{i=1}^{n} \log(\text{Antibody titer}_i)}{\text{Total Number of Animals in Sample (n)}}\right)$$

5) The 75th percentile point is related to the sample median and is calculated as the $\log_2$(Antibody titer) of the n(0.75)th ordered sample value/element. For example, consider a sample containing the SN titers 0 (negative), 4, 4, 8, 8, 16, 32 and 128. The 75th percentile point of this sample is the 6th (8 X 0.75) ordered element. The logarithm(base 2) of 16 is 4.

RESULTS

Participation

Producer participation in the Marshall county pseudorabies pilot eradication project (MCPPEP) was excellent. It was estimated that over 95%, 222 in total, of the known herds of swine were involved as of March 1, 1986. Approximately 80% of these farms were initially sampled during the first six months of the program.

Forty-six operators reportedly ceased raising hogs during the time period from the start of the project (July 1983) to March 1, 1986. The attrition rate was approximately 1.3 farms per month for the period of time. The frequency seemed to be unrelated to pseudorabies infection status. Based on the attrition rate and the cumulative number of participating farms, it was estimated that between 170 and 180 farms were actively involved in the project during most of its duration and up to the present time.

Serological Test Results

The results of initial testing indicate that pseudorabies (PR) infection was present in 32 of 222 total herds. Evidence of PR infection appeared in nine additional herds of swine that were initially classified as PR-free. Summaries of the infected herds are given in tables 4 and 5. Descriptions of some of the columns in the tables are neces-

sary. Column one is a random identification number assigned to each project herd. The number of breeding stock is the operator's estimated inventory of sows, boars and breeding-age gilts at the time PR infection was detected. The vaccine type corresponds to the PR vaccine type, if any, used prior to the initial herd samplings. The serologic parameters are based on serum neutralization test results of the first one to three samplings of the herd breeding stock. If no adult swine samples were available, they are based on progeny/feeder pig samplings. The calculations of these parameters are given in MATERIALS AND METHODS.

Approximately 23,460 serum samples were evaluated for PR-specific serum neutralizing (SN) antibodies (July 1983 through January 1986). Of these samples, significant level of PR-specific SN antibodies were detected in 3859 (16.4%). Antibody titers greater than or equal to four are considered significant. The overall seropositive rate tends to decrease during the progression of the project and is depicted in figure 3.

Farm Categorization

One of the 7 project plans was generally agreed upon with each operator. It became necessary to move farms from one category to another due to individual circumstances. For example, an operator decides to change herd plans by starting a PR vaccination program. The farm may have been

Table 4. Summary of Initial Pseudorabies Infected Herds: Marshall County Pilot Elimination Project July 1983 - October 1984

Herd	Number breeding stock	First evidence infection	Vaccine usage	Serologic parameters (Serum Neutralizing) Initial PR prevalence	Highest SN Titer	Geometric Mean Titer	75th percentile point	Elimination plan	Clean Up time (months)
4	115	Nov 83	None	4%	8	1.0	-	3B	6
5	52	Oct 83	None	4%	16	1.1	-	3B	10
6	187	Nov 83	MLV[a]	64%	128	5.3	4	3C	10
8	93	Sep 83	MLV	97%	256	10.2	5	3C	18
9	123	Oct 83	MLV	84%	64	4.9	3	3B	14
13	120	Jul 83	MLV	96%	256	10.2	4	3C	19
14	156	Jul 83	MLV	100%	128	24.3	5	3C	Out[b]
15	218	Jul 83	MLV	77%	32	4.0	3	3C	11
17	62	Sep 83	MLV	42%	128	3.4	4	3C	10
19	215	Oct 83	None	83%	64	7.6	-	3C	Out
22	130	Sep 83	K[c]	100%	128	25.1	5	3A	20
25	83	Oct 83	None	96%	256	13.5	-	3C	18
26	0	Jun 84	None	73%	32	3.2	-	3A	11
27	73	Jul 83	MLV	55%	64	3.5	4	3C	21
28	74	Jul 83	None	22%	4	1.2	-	3B	13
30	47	Jun 84	None	46%	64	5.4	-	3C	25

31	146	Sep 83	MLV	81%	128	8.3	4	3C	22
32	38	Jul 83	MLV	57%	128	4.9	4	3C	15
36	0	Jul 83	None	82%	256	9.0	-	3A	Out
38	84	Dec 83	MLV	62%	256	5.9	4	3C	27
41	103	Aug 83	MLV	51%	128	4.4	4	3C	17
42	0	Oct 84	None	75%	32	6.3	-	3A	5
44	80	Nov 83	None	28%	32	2.4	-	3C	8
45	330	Oct 83	K	75%	64	4.4	3	3C	14
47	140	Oct 83	MLV	69%	128	5.1	3	3C	16
49	41	Nov 83	MLV	12%	32	1.2	0	3A	10
57	78	Sep 83	MLV	79%	128	13.8	5	3C	-[d]
58	144	May 84	K	80%	64	8.1	4	3C	28
59	166	Apr 84	None	100%	64	9.5	-	3C	11
61	52	Aug 83	K	0%	-	-	-	3C	7
62	524	Aug 83	MLV	87%	128	7.5	4	3C	-
63	62	Sep 83	K	16%	16	1.3	0	3C	15

[a]Modified-live pseudorabies virus vaccine.

[b]Herds 14, 19, and 36 ceased hog raising operations before pseudorabies clean up was complete.

[c]Killed pseudorabies virus vaccine.

[d]Herds 57 and 62 remain pseudorabies seropositive as of March 1, 1986.

Table 5. Summary of Farms that Experienced Subclinical and/or Clinical Pseudorabies (PR) Outbreaks During the Marshall County Pilot Elimination Project (July 1983 - March 1986)

				Serologic parameters (Serum Neutralizing)					
Herd	Number breeding stock	First evidence infection	Vaccine usage	Initial PR prevalence	Highest SN Titer	Geometric Mean Titer	75th percentile point	Elimination plan	Clean Up time (months)
1	102	Apr 85	None	78%	16	3.5	-	3C	-[a]
3	155	Apr 85	MLV[b]	100%	32	11.0	5	3C	-
7	70	Jan 85	None	92%	64	10.2	-	3C	-
18	100	Oct 84	K[c]	80%	64	7.2	4	3C	-
21	64	Jan 85	K	100%	32	16.9	5	3C	-
34	124	Jan 85	MLV	82%	64	9.4	5	3C	-
43	100	Aug 85	None	3%	32	1.5	-	3B	1
50	450	Jul 85	K	92%	64	25.6	5	3C	-
172	0	Aug 85	None	-	-	-	-	3A	5

[a]Herds that remain infected as of March 1, 1986 (Herd numbers 1, 3, 7, 18, 21, 34, and 50)

[b]Modified-live pseudorabies virus vaccine

[c]Killed pseudorabies virus vaccine

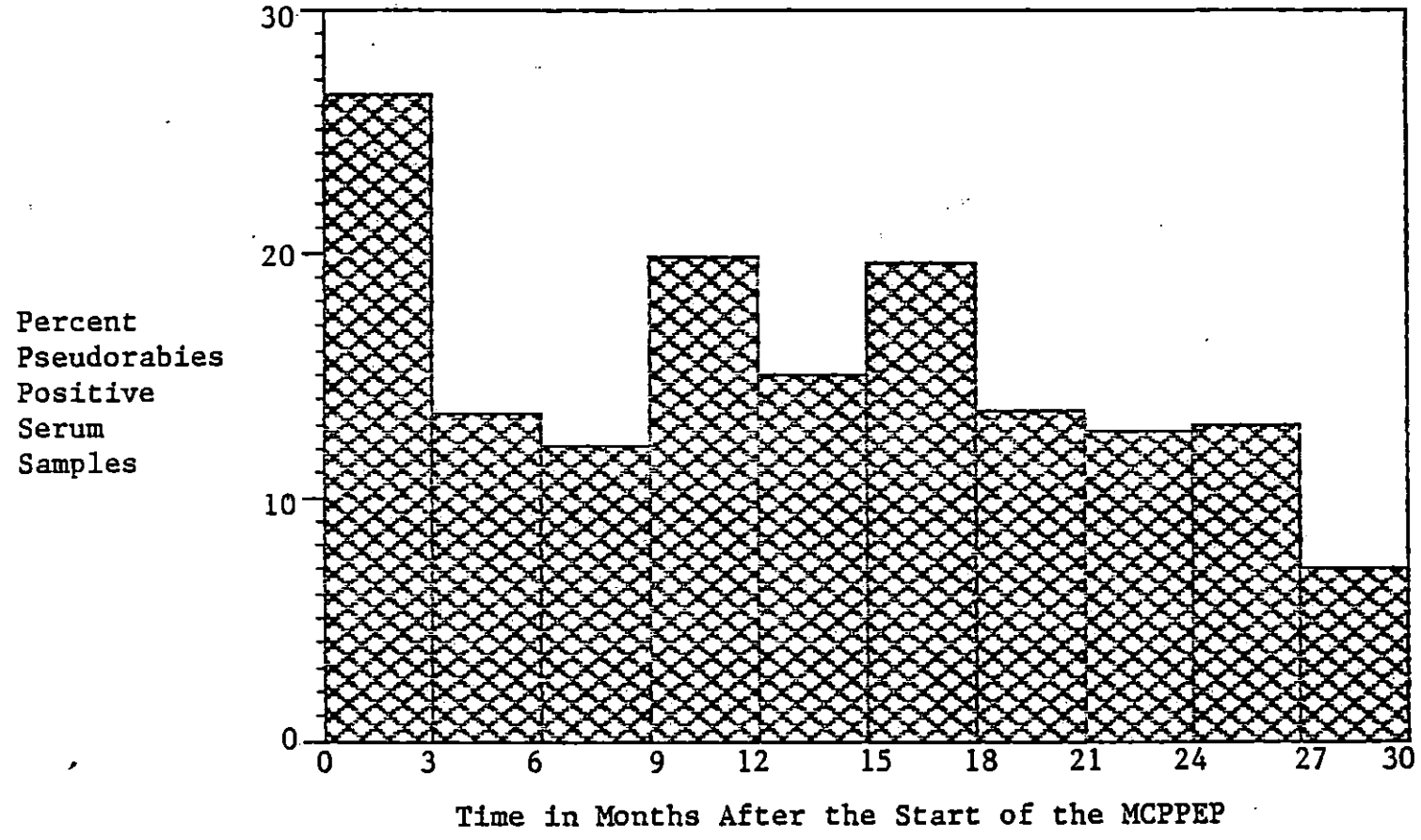

Figure 3. Summary of Serum Neutralization Test Results: Pseudorabies Seroprevalence in Marshall County, Iowa Herds of Swine (July 1983 to January 1986)

in herd plan 1A and now will be reclassified into plan 2A. The number of farms in each herd plan thus fluctuated over the course of the project. In January 1984 and January 1986, the number of project herds in each plan were as follows:

	January 1983				January 1986		
Herd Plans	1	10	Number of Farms	Herd Plans	1	9	Number of Farms
	1A	88			1A	66	
	2	5			2	5	
	2A	48			2A	82	
	3A	1			3A	2	
	3B	4			3B	0	
	3C	23			3C	13	
		179	Total			177	Total

Pseudorabies Clean Up

The pseudorabies clean up procedures used in the MCPPEP were very effective. Of the 32 originally infected herds, all were re-classified as PR free as of March 1, 1986 with the exception of two herds. The average time period involved in attaining PR free status was 401/27 = 14.9 months. Figure 4 displays the time table involved in this process. Three farms ceased raising hogs before sufficient evidence indicated they were free of the infection. These 3 instances were due to individual circumstances which appeared unrelated to the MCPPEP. Five of these farms elected to use test-and-removal (plan 3B), 30 used offspring segregation (plan 3C), and the clean up methods of the

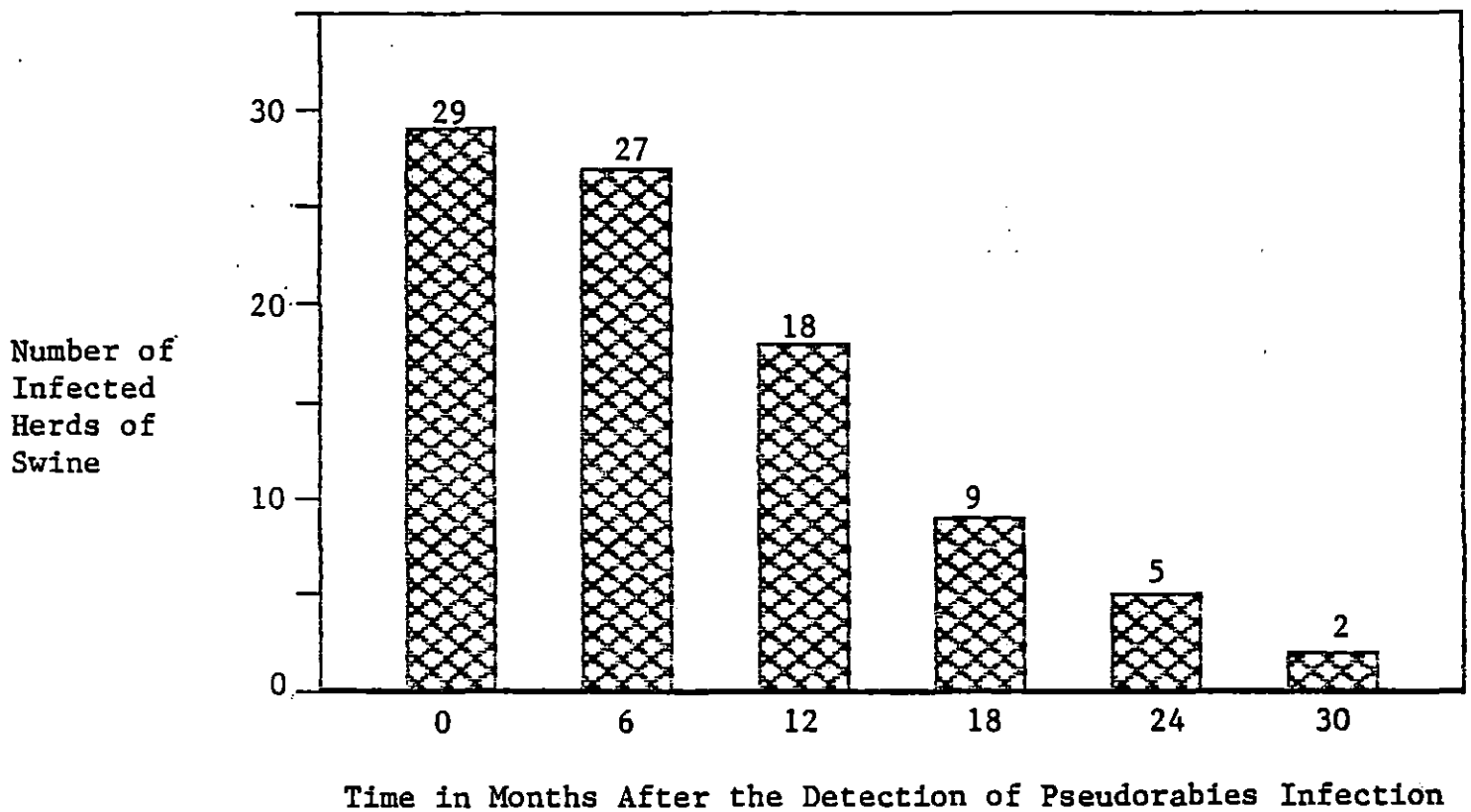

Figure 4. Pseudorabies Elimination Time Table: The Number of Initially Infected Herds of Swine[a] That Remained Infected as Compared with Time in Months After the Detection of Pseudorabies Infection

[a]There were 32 total herds -- Three ceased raising hogs before attaining PR free status.

DISCUSSION

The participation of the Marshall county pork producers is commendable. Credit goes to a well organized local pork producer association, Marshall county practicing veterinarians, state veterinarian of Iowa Dr. Merle H. Lang, the project veterinarian Dr. Roy Gallentine, individuals from APHIS and Iowa State University, but firstly the producers themselves. The cooperation involved in this project is at least one optimistic sign amid Iowa's troubled agricultural economy.

The "down the road" sampling procedure proved to be a successful method of detecting infected herds. One advantage of testing swine on their home premises is that there is less potential for identification mistakes. Slaughter sampling, the other plausible collection procedure, is useful only if positive swine can be traced back to the farm of origin. Historically, identification systems for swine have been unreliable. Down the road sampling offers another important advantage over slaughter sampling, especially in high prevalence areas. It can identify infected herds more rapidly. With slaughter sampling, a period time must be allowed for trace-back and then on farm testing. The delay in detecting infected herds could potentiate dissemination of pseudorabies infection. If infection is detected quickly, early precautions against virus spread can be

instituted. Also, rapport with the farmer would already be established. Based on the MCPPEP, the individual operators' understanding of their responsibility in preventing PR propagation and is the most important consideration in its elimination.

It is well-established that antibodies elicited by current PR vaccines cannot be directly differentiated from those due to field infection.[17] In this project, a detailed epidemiologic examination was made of each vaccinated herd. This examination was based on the results of serum neutralizing (SN) antibody titrations. The use of the highest antibody titer, as described earlier, successfully identified the infected herds. The geometric mean antibody titer of the breeding stock showed a general relationship to antibodies resulting from PR or from vaccination but was so variable that definite conclusions could not be reached. A more useful parameter of PR infection was the 75th percentile (Q75) point. A Q75 value of 4 or higher was indicative of PR infection. Based on data from Marshall county herds of swine, the Q75 point is less variable than the highest antibody titer. Both methods reliably detected infection in vaccinated stock.

Killed virus preparations were the only pseudorabies vaccines used in the MCPPEP. Modified-live products were reserved for use in the face of a clinical outbreak. No

clinical disease was reported in infected herds which vaccinated with the killed virus product. A new type of vaccine has also been utilized on a limited basis. Three field trials were conducted in two Marshall county herds with the subunit vaccine developed by Platt et al.; the publication of results is forthcoming.

A shift in the number of herds in vaccination categories can be noted from January 1984 to January 1986. As the project progressed, more farmers initiated a PR vaccination program. It is interesting that the prevalence of PR specific antibodies decreased during the same period of time. In our experience, serum neutralizing antibody titers induced by field vaccination with killed PR vaccine last from 2 to 3 months. Modified-live vaccines seem to induce much longer lasting antibody titers. It is felt that the use of killed vaccine in the MCPPEP facilitated the interpretation of serologic data. Some may argue that the killed vaccine is less efficacious but there is no solid evidence to support this belief.

Nine farms developed evidence of pseudorabies infection after their categorization as infection free. The source of the infection could be determined in all but 2 cases based on circumstantial evidence. Four herds were thought to have acquired the virus through feeder pig and/or bred gilt purchases from herds of unknown status (outside the county).

It was speculated that three other herds contracted the infection by means of their close contact with an infected herd in a neighboring county. Possibilities for the source of infection to the last two herds include: purchased breeding stock, infected wildlife, and returning stock to the premises after loaning them or in some other way exposing them to the infection.

It is anticipated that pseudorabies infection will be eliminated from these herds before 1987. Four are close to achieving PR-free status as of March 1, 1986.

CONCLUSIONS

The Marshall county pseudorabies pilot eradication began in the summer of 1983. The results thus far indicate that PR infection can be eliminated from infected swine farms and probably from an endemic geographic area as well. Evidence indicates that the infection has been eliminated in all but two of 32 initially infected herds. A brief description of the situation on each of these two farms is appropriate.

Farm 62 is a large farrow to finish confinement operation. The herd has a history of PR dating back several years. The operator had agreed to follow the basic cleanup methods of herd plan 3C (offspring segregation) in 1983. As of March 1, 1986, little has been done toward following the recommended cleanup procedures. Communication has been maintained with this operator and every effort has been extended to work out a viable solution for eliminating the infection. Numerous visits with the farmer have seemed fruitful and yet offspring segregation has not been attempted.

Herd number 57 is also a farrow to finish operation. The operator is a relative of the operator of herd 62. Pigs have been moved between the two farms in the past. It is unclear whether clean up procedures have been followed. Communication is maintained with this farmer and it is hoped

that the infection can be eliminated in the near future.

Overall, it was not possible to compare the relative effectivity of the different methods of PR elimination. Each methods was used in different circumstances. Offspring segregation procedures were utilized in 73% (30/41) of infected Marshall county herds. True depopulation/repopulation techniques were only used twice. The cost of depopulation/repopulation was many times the costs of the other two cleanup methods. An economic assessment of the MCPPEP is expected to be complete in April 1986. The costs of this pilot elimination program will be available after that time and may determine the future of pseudorabies control in Iowa.

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GENERAL DISCUSSION

Pseudorabies (PR) continues to be a health problem of swine in the United States. The annual cost of the infection approaches 20 million dollars. Federal pseudorabies regulations that were adopted in 1979 have not decreased its prevalence. Iowa has the highest prevalence of the major swine producing states.[46] Iowa also raises more hogs than any other state -- about one-forth of the total pigs in the United States.[47] The effects of the pseudorabies problem should be noted in Iowa if anywhere.

The costs of pseudorabies to Marshall county swine producers have been evaluated by members of the departments of Veterinary Preventive Medicine and Agricultural Economics at Iowa State University, Ames, Iowa. Results of these studies are expected to be released in the very near future. Measuring the effect of subclinical PR on herds of swine has been a difficult task. One of the reasons for this is that many farmers don't keep detained enough records for such an assessment.

In the Marshall county pseudorabies pilot elimination project (MCPPEP), it was noted that farms with PR infection report more hemophilosis than PR-free farms. It is not clear what factors may be involved. Perhaps the prevalence of Hemophilus infection is similar in the two groups and yet the clinical severity is notably different. It is not clear

whether farm management factors or disease factors may be involved. It is possible that concurrent PR infection may exacerbate the severity of Hemophilus pneumonia. Farm management conditions/factors that may be involved include:

a. Procedures used when new stock are introduced to the herd.

b. Frequency of acquiring new stock.

c. Extent of confinement.

d. Extent of crowding, temperature fluctuations and other stresses.

e. Disease awareness and control.

f. Use of antibiotics in livestock feed.

These factors may be involved in both severity and prevalence of PR and Hemophilus infections. Further epidemiologic studies are indicated.

In SECTION I, we attempted to study other infections occuring as sequelae to PR infection. Salmonella choleraesuis var kunzendorf and Hemophilus pleuropneumonia infections subsequent to PR infection were studied. These two bacterial infections are extremely common in Iowa swine. Salmonellosis is characterized as a gastrointestinal infection. Affected animals often display systemic signs of disease as well. Clinical salmonellosis is most often noted in pigs from 30 to 150 pounds in size. Hemophilosis is primarily a respiratory infection. The acute form of

hemophilosis is characterized by necrohemorragic pulmonary foci. The infection seems to be more severe in confinement situations and can be devastating to herds of swine.

The experimental work outlined gave no indication that salmonellosis and hemophilosis were exacerbated in pseudorabies convalescent pigs. There was evidence, however, that various immune system parameters are altered for a period of time during and shortly after PR infection.

When killed antigens were administered to PR convalescent swine at different times after PR inoculation, no depression of humoral responses was noted. In fact, the humoral responses were significantly increased to a killed Brucella abortus str. 19 preparation which was given 5 days after PR inoculation. One of the possible explanations for this is that PR infection may stimulate the release of certain lymphokines, for example the interleukins, which tend to augment the immune response. Lymphokines are soluble mediators of the immune system. Results of another study indicate that humoral immune responses to a modified-live virus (MLV) transmissible gastroenteritis (TGE) were significantly depressed in PR convalescent pigs. The results are similar to those experienced by Tiawanese researchers with a modified-live virus hog cholera vaccine.[25] It seems that subsequent to PR infection, humoral responses to certain modified-live virus preparations are depressed. It is

unclear for what period of time after PR infection this effect can be observed. In our study with TGE vaccine the pigs were vaccinated twice, 34 and 48 days after PR inoculation. The mechanism for this depression may be either a direct or indirect affect of the virus on the humoral immune system. A direct affect is unlikely based on the results of our work with killed antigens. Possible indirect affects of PR infection on the humoral response to these vaccines include viral interference mechanisms such as interferon induced interference.

There is evidence that PR infection depresses certain parameters of cell-mediated immunity (CMI). Depression of lymphocyte blastogenic (LB) responses to the mitogens phytohemagglutinin, pokeweed mitogen, and conconavalin-A were noted during and shortly after PR infection. One month after PR inoculation the CMI parameters had returned to levels that were similar to normal control animals. It is apparent that if PR convalescent pigs are immuno-compromised, the effects are subtle and are probably evident only during acute stages of the infection and possibly a few weeks thereafter.

One practical implication of these results relates to the use of swine vaccines. One may expect modified-live virus vaccine failures in pseudorabies infected herds. As far as I know there are only three viral swine vaccines com-

monly used in the midwest United States: pseudorabies, TGE, and porcine parvovirus vaccines. Further research is needed to determine if MLV pseudorabies vaccine depresses humoral immune responses in a similar manner. It would be prudent to avoid administering MLV PR vaccine at the same time as other MLV vaccines.

As mentioned earlier, measuring subclinical PR can be a difficult undertaking. Under our experimental conditions there was no evidence that Salmonella and Hemophilus infections were more severe in PR-infected pigs as compared with PR-free control animals. Other infections, or the same infections under different conditions, may be exacerbated by pseudorabies. One small study from the University of Minnesota indicated that PR and Pasteurella multocida may act synergistically in the induction of bronchopneumonia.[15] Gustafson noted decreased conception rates in herds of swine after PR outbreaks. Presumably this was due to bacterial metritis secondary to PR infection.[18]

Probably the best way to measure the clinical effects of other infections subsequent to subclinical PR infection would be to artificially introduce the PR virus into experimental herds in a double blind fashion. Since such research is not practical, we resort to field reports before and after the introduction of PR to a herd. We then extrapolate to obtain a general picture of the costs of subclinical PR.

It is hoped that the costs can be accurately determined in the upcoming national economic assessment of pseudorabies. Various researchers, including some from Iowa State University, will be addressing this and other questions.

The costs of pseudorabies become important when considerations are made to control the infection. The higher the costs of subclinical PR and sequelae thereto, the more economically justified eradication becomes. Economic considerations are paramount, especially in the present condition of midwestern United States agriculture.

The Marshall county pseudorabies elimination project (MCPPEP) has been an example of successfully eliminating PR infection from a endemic geographical area. Out of 32 herds found to initially infected with the virus since the start of the project (July 1983), only two remain so as of March 1, 1986. Both offspring segregation and test-and-removal provided low cost PR elimination from the Marshall county herds. Depopulation/repopulation was not necessary in any of the herds but was the choice of two operators. It proved to be a costly method of clean up. One of the remaining infected farms in the project is a large confinement operation. A pseudorabies clean up procedure was agreed upon with the operator of this farm early in the project but offspring have not been segregated as recommended as of March 1, 1986.

Some have contended that PR cannot be eliminated from large confinement swine producing operations unless the herds of swine are completely depopulated. Certainly more experience with such herds is desirable. The experience thus far with the one large operation in the MCPPEP is no indication that PR will never be eliminated from such operations without depopulating. Depopulation is not an option unless a fair reimbursement system appears on the scene. Many of the Marshall county herds were small in comparison with such large corporate operations but perhaps there is no difference in PR cleanup difficulty between small and large swine raising operations. It may very well be an attitude difference. Perhaps large corporate-based operations are more concerned about the inconveniences of PR cleanup and less concerned about their responsibility to the industry as a whole. They seem to be more concerned about the potential economic benefits involved. If no benefits are noted there seems to be little incentive to change.

Let us consider the effect of current pseudorabies regulations on producers. The quarantine procedure in the MCPPEP was unique. The usual procedure is to quarantine herds determined to be infected by any method. This quarantine stipulates that pigs may only be sold to slaughter. Its real impact is on producers who sell seed stock to other farmers. When their herds are determined to be infected,

there is a sudden shock to that business. All swine that had previously been sold to other producers as replacement stock must now be sold at slaughter prices. This may mean a 50-60% decrease in cash flow, even if the producer has facilities to raise swine to market size. If the operators do not have sufficient facilities to raise the pigs they must cease producing or find other quarantined premises to finish them. All of this will continue unless PR seropositive swine are removed from the premises and the herd is subsequently declared free of the infection. Our experience from the MCPPEP is that this takes an average of about 15 months.

This relatively small group of swine producers, the seed stock producers, are unfairly affected by the current regulations. The rest of the swine producing industry goes about its business vaccinating whenever appropriate. Other PR-free swine producers supply seed stock as needed. Yes, quarantines are administered to these other operators also. On a practical level, however, business continues as usual. Swine go to slaughter as before. A routine pseudorabies vaccination schedule may be the only difference.

The disease affects more than swine producers -- the infection can occur in almost every domestic animal species and is invariably fatal. I maintain that it isn't entirely up to the swine industry to decide. The sun may burn out

while we wait for a united PR policy from the swine industry. Let us examine the options -- there are three main ones.

We could maintain the status quo. The problems outlined would continue but the anxiety of change would be avoided. State and federal veterinarians would continue to issue quarantines to infected premises but would not have sufficient funding or authority to initiate PR clean up procedures.

Another option would be to relax or remove current regulations. Allow PR infected pigs to move freely in the sale channels. The clinical aspects of the infection would be controlled by vaccination. Quarantines would be forgotten about and no new ones would be issued. In my opinion this option is totally unrealistic. If a producer has the option of purchasing known PR infected pigs versus known PR free at the same price, which will he buy? The group that are free of pseudorabies of course. The PR infection status of most herds will become well known in the future because veterinarians will continue to test swine as a matter of assessing herd health. Or should we ban the use of the serologic tests for pseudorabies antibodies? Certainly not.

The last option is to consider eradicating the infection from the United States or at least giving more substance and fairness to current control regulations. Many

states have already developed an eradication attitude toward pseudorabies. Regulations would need to be instituted on a state by state basis. Some European countries are currently in the final stages of PR eradication including England and Denmark. The following facts about pseudorabies make the infection amenable to eradication:

a) Swine are the only known reservoir hosts of the virus.

b) The virus is relatively easily inactivated by ultraviolet light, desiccation, heat and commonly used disinfectants.

c) The infection can be successfully eliminated from herds of swine using the technology and procedures we already have and are using.

d) The scientific community knows more about pseudorabies than it knew about any other animal disease that it successfully eradicated from the United States.

Many of the things learned in the Marshall county pseudorabies pilot elimination project about the epidemiology of pseudorabies are things that we already suspected. Pseudorabies can be eliminated from herds of swine using low cost procedures. Also, to control the spread of infection one needs to control the movement of infected swine. A few new concepts have gained recognition as well.

A sampling approach to serological testing has been an effective method of identifying infected group of pigs. Instead of serologically testing each pig, a sample based on

the total number is used. This method provides a high probability of detecting infection at minimal cost.

On farm testing of swine by area practicing area veterinarians was utilized in the MCPPEP. It provided a quick determination of the infected areas of the county. Most of the infected herds were located in the first 6 months of the project. Slaughter sampling techniques could not offer this kind of turn around time especially with the problems currently experienced with swine identification at slaughter plants. Detecting PR infection in vaccinated herds was possible based on SN antibody titer levels in the breeding stock and the detection of antibodies in market weight progeny. The evaluation of PR infection in these herds was also facilitated by evaluating the results of samples obtained over a period of time; infected and suspect herds were sampled every 4 months.

The main contribution of the MCPPEP to our knowledge of pseudorabies is the people aspect. Cooperation would be necessary at all levels of an eradication program. Producers must be in favor of efforts to eliminate the infection. Local practicing veterinarians are the link between producers and the scientific and regulatory world. They must be knowledgeable and objective in regard to pseudorabies control. Administrators of such a program must listen to and work directly with farmers. They must be well

organized; computer-based record keeping systems would be extremely helpful in this regard.

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