



PRRS Control And Elimination Toolkit

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PRRS Control and Elimination Tool Kit

Introduction

The PRRS Control and Elimination Tool Kit is a resource for veterinarians to utilize in the control and elimination of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) from a particular production site or a specific population of pigs. The tools are based on the current knowledge that is available from scientific research, field trials and protocols that are used on farm. Knowledge is not static and the reader is urged to keep up with new information as it arises.

On numerous farms throughout North America and indeed the world, elimination of PRRSV from individual herds has been achieved with a variety of protocols. In the scientific literature, some authors reported success rates up to 91-100% in elimination of PRRSV from herds, especially from breeding herds (Dee et al, 2001; Dubois, 2007).

The tool kit is divided into three main sections.

- a. The **“PRRS Control Programs”** with a first subsection on **“PRRS Immunity Building Tools”**. These are tools that are used to build a protective level of immunity in the pig population. When all pigs possess protective levels of immunity, the PRRSV will be unable to maintain replication and will be eliminated from the population.

The second subsection titled **“PRRS Challenge Reducing Tools”** lists management tools that are used to reduce the challenge dose of the virus that is available to infect a susceptible animal. Less virus in the environment leads to fewer infected animals and lowers the farm prevalence rate to the point where elimination programs can be initiated.

- b. The **“PRRS Elimination Programs”** describe various programs that are used in PRRSV elimination. Each of these programs requires that immunity is maximized and challenge levels minimized and reinfection prevented. As such, the success of these programs requires effective use of the PRRS immunity building and challenge reducing tools.
- c. The **“PRRS Monitoring Tools”** subsection provides guidelines on how to detect PRRSV infection and to monitor the success of a PRRSV elimination program that is being used in a given herd.



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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Control Programs

1. Porcine Reproductive and Respiratory Syndrome (PRRS) immunity building tools

1.1 Homologous versus Heterologous Immunity

Homologous immunity is the protection generated by the pig's immune system towards a strain of PRRSV that the pig has previously been exposed to. Homologous immunity is generally more effective than heterologous immunity (Lager et al, 1999). The homologous protective immunity after PRRSV exposure may persist for the production life of the animal (Lager et al, 1997). It has recently been shown that for PRRSV, homologous immunity is not 100 percent effective and animals that are re-exposed to the same virus strain may still develop a viremic infection (Murtaugh and Wagner, 2010). It is however, accepted that homologous immunity is more protective than heterologous immunity. (Lager et al, 1999).

Heterologous immunity is the protection generated by the pig's immune system towards a strain of PRRSV that the pig has not previously been exposed to. Heterologous protection is variable against challenge with genetically diverse strains. Heterologous protection, including the protection derived from live attenuated vaccine is demonstrable even against highly virulent strains (Murtaugh, 2009). Protection against infection with a PRRSV strain that is not exactly the same as the strain that the animal has been immunized with is called "cross-protection". The level of cross-protection is probably related to the genetic similarity of the antigens presented by the strains that are involved in the cross-protection. To date, the exact identity of the genes that code for structures in the virus that stimulate immunity remain unclear (Murtaugh et al, 2004; Murtaugh, 2009).

1.2 Commercial Modified Live Virus PRRSV Vaccines

Commercial Modified Live Virus (MLV) PRRSV vaccines have been used in control and elimination programs with variable success (Dee et al, 1998; Thomas et al, 2009; Dee and Philips, 1998; Cano et al, 2007; Gillespie and Carroll, 2003). MLV PRRSV vaccines are usually effective in reducing clinical disease following a challenge with field isolates of PRRSV but they are usually not as effective in protecting against viral infection (Okuda et al, 2008; Cano et al, 2007). The level of heterologous protection conferred by MLV vaccines is variable from one field challenge isolate to another (Opriessnig et al, 2005). MLV vaccines have difficulty in sustaining immune protection to heterologous strains in sows compared to natural homologous protection (Kimman et al, 2009; Lager et al, 1997). The degree of genetic homology of ORF5 between the MLV vaccine and challenge isolate is not a good predictor of vaccine efficacy (Prieto et al, 2008; Opriessnig et al, 2005). While MLV vaccines do shed in naïve populations, transmission has not been detected following re-vaccination (Dee, 2004; Gillespie and Carroll, 2003). The use of MLV vaccines is discouraged in negative herds because of potential shedding (Dee, 2004), and it can



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create diagnostic confusion as it is difficult to distinguish between vaccine virus and field virus infections.

1.2.1 Replacement Gilt/Boar Vaccination

Replacement gilts and boars that are not immune to the specific PRRSV that is circulating in the breeding herd are at risk of infection after entry to the herd (Dee, 2004). Gilts that are infected with PRRSV after entry to the breeding herd will shed PRRSV (Dee and Philips, 1998). PRRSV infection of pregnant gilts and especially those gilts that are in the last trimester of pregnancy may result in vertical transmission of PRRSV from the gilt to her piglets (Cano et al, 2010; Zimmerman, 2007). Vaccination of replacement females and boars with commercial MLV PRRSV vaccine prior to entering a breeding herd that has a circulating field strain of PRRSV will reduce the probability of infection and shedding (Benson et al, 2000). The reduction of the risk of PRRS will be proportionate to the level of cross protection that the vaccine provides to the field strain of PRRSV that is currently circulating in the herd. Results of vaccinating gilts with commercial MLV PRRSV vaccines are variable as not every PRRSV strain is totally controlled by commercial MLV PRRSV vaccine-induced immunity (Opriessnig et al, 2005).

1.2.2 Breeding Herd Vaccination

Commercial MLV PRRSV vaccine may be used to provide mass exposure to the sow herd (Dee et al, 1998; Dee and Philips, 1998; Gillespie and Carroll, 2003). Results may be variable because not every PRRSV is controlled to the same degree by vaccine-induced immunity (Opriessnig et al, 2005). Administering a second dose one month after the initial vaccination has been reported to prevent the spread of vaccine strain and field strain virus from lactating sows to piglets (Dee and Philips, 1998). For sows in the third trimester of gestation (66 to 114 days), vaccination may be delayed until after farrowing with the first vaccination occurring on day 7 of lactation and the second 30 days later (Dee et al, 1998). Simultaneous mass vaccination is beneficial as all susceptible animals are immunologically stimulated at the same time. When all sows in the herd have received two doses of vaccine, the herd can be closed, and as the flow of pigs is controlled the breeding herd can be cleared of the PRRSV infection because no reservoir of susceptible animals is available to undergo acute infection and shedding of PRRSV (Dee et al, 1998; Dee and Philips, 1998; Gillespie and Carroll, 2003). The extent and duration of protection against heterologous PRRSV infection is variable and is dependent on antigenic relatedness between the commercial MLV PRRSV vaccine and the field strain infection in the herd (Lager et al, 1999).

1.2.3 Growing Herd Vaccination

Commercial MLV PRRSV vaccine may be used to provide mass exposure to the growing pig population (Dee and Philips, 1998; Gillespie and Carroll, 2003). Results of vaccinating growing pigs with commercial MLV PRRSV vaccines are variable as not every PRRSV strain is totally controlled by commercial MLV PRRSV vaccine-induced immunity (Opriessnig et al, 2005). Vaccination of the growing pig should ideally be delayed until passive immunity has waned because passively acquired immunity might interfere with vaccine efficacy (Benfield et al, 1999). Because the timing of PRRSV circulation and pig exposure varies by farm and management style, it is very difficult to provide a universal recommendation for vaccination timing. The protective immunity provided by commercial MLV PRRSV vaccine is slow to develop and vaccination

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should be timed before virus exposure (Benfield et al, 1999). Opriessnig et al reported that commercial MLV PRRSV vaccines can provide heterologous immunity in growing pigs if used 5 weeks prior to expected exposure (Opriessnig et al, 2005). This allows enough time for the pig's immunity to build up before exposure. Cross-protection may be improved if the vaccine is boosted one month after the initial vaccination (Dee and Philips, 1998). Challenge trials have demonstrated the efficacy of commercial MLV PRRSV vaccines in reducing the impact of exposure to heterologous strains of PRRSV in pigs while field trials have demonstrated the cost benefits of commercial MLV PRRSV vaccines in commercial pigs (Opriessnig et al, 2005; Mengeling et al, 2003; Schuon et al, 2008; Desrosiers, 2000). The effectiveness of a commercial MLV PRRSV vaccine in pigs that were previously infected with a heterologous PRRSV strain was tested in growing pigs (Cano et al, 2007). The commercial MLV PRRSV vaccine was administered starting at 1 week post infection. The vaccine was effective in reducing the duration of viral shedding. MLV PRRSV vaccines can be used in a partial depopulation in order to reduce the risk of shedding in the older pigs that remain in the barn (Dee and Philips, 1998; Gillespie and Carroll, 2003). Mass vaccination using commercial MLV PRRSV vaccine and unidirectional pig flow in a PRRSV positive growing pig population combined with partial depopulation has been shown to successfully eliminate PRRSV (Dee and Philips, 1998; Gillespie and Carroll, 2003).

1.3 PRRS Field Virus Exposure

1.3.1 Rationale and principles

The concept of using exposure to a live field virus is as old as the science of vaccination. This technique is not novel by any means and has been used for control of other viral diseases such as enzootic transmissible gastroenteritis (Moxley et al, 1993). It is based on the principle that homologous immunity is generally more effective than heterologous immunity (Lager et al, 1999). Mass PRRS field virus exposure is used to ensure 100% exposure of all animals to a specific PRRSV field strain in order to produce a uniformly seropositive herd and prevent the development of subpopulations of susceptible animals (Ruen et al, 2007; FitzSimmons, 2005). It is important to ensure that the PRRSV strain that is used in the exposure of a population within a barn is actually taken from within that building site. For example, if a sow herd is to be exposed to field type PRRSV then the virus should be taken from the sow herd and not from an off-site nursery (FitzSimmons, 2005) which may be circulating another PRRSV. Following this rule will minimize the chances of inadvertently introducing a new field virus to the breeding herd from another site.

Planned exposure of field virus strains to naïve animals usually produces clinical signs that are commensurate with the virulence of the PRRSV strain being used (FitzSimmons, 2005). For any individual animal the resulting infection will have the same severity as the natural field infection. Through the use of planned exposure we are able to influence the timing of the infection and thus be able influence the stage of reproduction at which animals are exposed and the course of spread of the virus through the barn.

The potential risk of concurrently spreading other pathogens at the same time as the planned exposure to field strains of PRRSV as well as any potential liability issues must be taken into account. (Corzo et al, 2010; O'Rourke, 2005). Major reproductive losses have been reported after the use of planned exposure of pregnant sows to field strains of PRRSV (Bruner, 2007). The same



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author reported reproductive losses even after the second planned exposure of pregnant sows to the same PRRSV field strain (Bruner, 2007). With on-farm serum inoculation, there can be a risk of cross contamination during preparation of the serum inoculate in the laboratory (FitzSimmons, 2005).

Planned PRRSV field isolate exposure can be used in:

- gilt and boar acclimation (Hill et al, 2004; Batista and Dee, 2002; Batista et al, 2002)
- whole or partial herd exposure during an outbreak (Hill et al, 2004; Ruen, 2003)
- whole or partial herd exposure in herds that occasionally produce viremic pigs at birth (Pittman, 2007; Ruen et al, 2007)

1.3.2 Sources of infective PRRSV field virus for Live Virus Exposure

Serum Injection:

The PRRSV strain specific to the population of pigs is harvested by collecting serum from pigs that have PRRSV circulating in their blood stream (FitzSimmons, 2005). Protocols for serum collection, serum storage and on-farm live PRRSV inoculation have been published (Hill et al, 2004; Pugh et al, 2005; FitzSimmons, 2005; Ruen, 2003). Serum injection has the advantage of assuring 100% exposure to all animals if the procedure is well executed by farm employees (FitzSimmons, 2005). Generally, weak born and clinically sick piglets from the farrowing barn provide serum with the highest virus concentration (Ruen, 2003). An infective dose ranging from 7 live virus particles (VP) per pig to 247 live VP particles per pig have been shown to be effective for inducing seroconversion in the population exposed (Pugh et al, 2005). Although live virus homologous serum injection can provide substantial protection against reproductive PRRS, a recent trial showed that it does not prevent transmission to piglets (Murtaugh and Wagner, 2010).

Tissue Feedback:

The PRRSV strain specific to the population of pigs is harvested by collecting tissues from pigs infected with PRRSV (Desrosiers and Boutin, 2002). These tissues are then fed back to the pigs that need to develop immunity (Dufresne, 2003). The problem with this method for PRRS exposure is that it is not entirely reliable as it is difficult to quantify the uniformity of the amount of live virus in the material that is used. (Hill et al, 2004). In the scientific literature, this method does not seem as widely used and reported as serum injection.

Shedding Pigs:

Pigs that are shedding the PRRSV strain specific to the selected pig population are identified. The shedding pigs are then placed in nose to nose contact with pigs that need to develop immunity. This method of exposure relies on both adequate viral shedding and contact between pigs and therefore is not entirely reliable (Hill et al, 2004). During an acute outbreak in a sow herd, aborting sows that are shedding virus can be moved throughout the sow barn in order to provide exposure to other sows (Desrosiers and Boutin, 2002). Usually, the duration of infection and the proportion of pigs persistently infected with PRRSV is higher in piglets than in adult animals (Batista and Dee, 2002; Ruen, 2003). Therefore, exposing replacement gilts and boars to young growing pigs that are shedding the PRRSV should be a more effective approach. A more reliable approach could



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be the exposure of naïve seronegative pigs to 6-week old pigs inoculated with the endemic PRRSV strains (Vashisht et al, 2008).

1.3.3 Mitigating The Negative Effects of Live Virus Exposure:

Deliberate exposure of pigs to virulent field type PRRSV can result in clinical signs, death or reduced sow herd productivity (FitzSimmons, 2005; Bruner, 2007). This presents some ethical dilemmas for those that contemplate the use of live virus exposure. The long-term scenario of successful PRRSV control or elimination may outweigh these concerns (FitzSimmons, 2005). There are tools that can be used to mitigate the negative effects of field virus exposure. Antifever drugs such as acetylsalicylic acid (ASA) and antibiotics such as tilmicosin can make the animals more comfortable as well as reducing death loss and reproductive losses. Unfortunately the results are variable (Misener et al, 2006; Nemechek et al, 2009; Batista et al, 2009; Fano et al, 2005; Pittman, 2007).

1.3.4 Exposure strategies for the different production categories

1.3.4.1 Acclimation of the Replacement Gilt and Boar

1.3.4.1.1 Replacement Animal selection

Ensuring that the source of your replacement breeding stock presents no risk of introducing new PRRSV strains or re-introducing existing PRRSV strains is a fundamental requirement of any successful PRRS control or elimination program. When sourcing replacement animals from an off-farm source it is highly recommended that only sources that can provide naïve seronegative animals be used (FitzSimmons, 2005). These sources must provide the producer and herd veterinarian with documentation of a history of routine testing of sufficient animals and outlines of herd disease control and biosecurity assessments to establish a confidence in the source herd status. The biosecurity of animal transport should also be included in this assessment. This also applies to all sources of boar semen.

1.3.4.1.2 Acclimation process

The acclimation process is composed of three periods : pre-exposure period, exposure period and post-exposure recovery period (Dee, 2004). The length of each period will vary from one herd to the other depending of the method chosen for acclimation and the status of the breeding herd.

Pre-exposure period:

The intent of a pre-exposure period is to provide the opportunity to verify PRRSV status of the incoming animals. In order to accurately assess very recent PRRSV infection in the source farm as well as infection during transport, replacement animals should be sampled serologically 14 days or later after arrival. If PRRSV status is required earlier than 14 days then a combination of serology and PRRSV PCR testing can be used. The replacement animals may have seroconverted to PRRSV if infection occurred at the source barn more than 14 days prior to delivery. Serologically testing earlier than 14 days post-entry can therefore give an indication of infection that occurred at the source barn. The PRRSV PCR test may also demonstrate the presence of viremia that resulted from infection at the source

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barn or in transit. Testing earlier than 14 days may allow for an early opportunity to ship the infected replacement animals. (Charbonneau, 2010).

Exposure period: The length of this period will vary accordingly to the technique chosen. Exposure by serum injection will be done in 1 day, MLV vaccination with 2 doses given 1 month apart will require 1 month, and natural exposure to infected pigs might require up to 60 days (Dee, 2004; FitzSimmons, 2005; Gillespie and Carroll, 2003).

Post-exposure recovery period: The goal of gilt and boar acclimation programs is to ensure that gilts and boars do not shed virus upon or after entry into the breeding herd. If PRRS acclimation is required, there should be at least a 90 day post-exposure isolation period after PRRS exposure before gilts and boars enter the breeding herd (Batista and Dee, 2002). This will allow for PRRSV shedding to stop prior to allowing these animals to enter the breeding herd. As the duration of the post exposure isolation period is increased, the risk of shedding of PRRSV after entry to the breeding herd is reduced (FitzSimmons, 2005). PCR testing prior to moving the animals into the main sow herd is sometimes done in the field. It is important to remember that a positive PRRSV PCR test is a strong indicator that an individual animal may be shedding virus. A negative PRRSV PCR test does not guarantee that an individual animal is not infective.

1.3.4.1.3 Acclimation site

On-site Acclimation In The Growing Herd: Purchased weaner gilts and boars may be introduced into the existing nursery or grow/finish pig flow at 5 to 45 kg (Batista and Pijoan, 2000). This method of acclimation may only be used where there is active and consistent PRRSV circulation in the growing herd. As mentioned before, this method of exposure relies on both adequate viral shedding and nose-to-nose contact between pigs and therefore is not entirely reliable (Hill et al, 2004). If some gilts or boars fail to develop immunity there will be an increased risk of PRRSV outbreaks in the breeding herd. If the PRRSV strain circulating in the off-site nursery or grower-finisher is different from the breeding herd, then there is a risk of introducing a new strain into the sow herd (FitzSimmons, 2005). This method of PRRSV acclimation is at best a short term program for PRRSV acclimation given that the ultimate goal is to eliminate PRRSV circulation in the growing pig population.

On-site Acclimation Facility In Closed Herds: In a PRRSV positive closed herd that is producing its own replacement breeding stock there will be some occasional PRRSV circulation. Replacement gilts and boars should be acclimatized to the PRRSV strains that are present in the herd but should not be shedding PRRSV upon or after entry to the breeding herd (Dee, 2004). An on-site isolation facility and PRRSV acclimation program can be used to allow for PRRSV exposure without putting the remainder of the herd at risk of exposure to excessive amounts of PRRSV. The use of an “on site” acclimation facility that is built close to, or directly attached to, the main unit will increase the risk of aerosol transmission of virus from the acclimation barn to the main unit. There must be strict adherence to biosecurity between the isolation / acclimation unit and the main barn (Torremorell et al, 2000). At minimum a change of clothes and boots followed by hand

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wash is recommended when moving between acclimation pigs and the rest of the herd. Ideally contact with the PRRSV exposed animals occurs as the last chore in the workday. All-in/all-out pig flow practices in the acclimation facility are recommended so that viral mutation and genetic drift are minimized (Dee, 2004).

Off-site Acclimation Facility: An off-site gilt acclimation facility affords maximum protection for the breeding herd in that aerosol transmission from the acclimation facility to the breeding herd is minimized and human flow and decontamination can be better controlled. This may be important with some strains of PRRSV that have a greater ability to travel by aerosol. In a recent study, aerosol transmission was demonstrated by the collection of infectious virus particles of a PRRSV strain as far as 9 km from the source farm (Otake et al, 2010). The off-site acclimation facility must meet or exceed the biosecurity requirements of the sow unit (FitzSimmons, 2005). An off-site acclimation facility that is located too close to other farms may increase the risk of infection of the replacement animals to other PRRSV strains via flies, mosquitoes, aerosol etc (Zimmerman, 2007). Air filters can be installed to reduce the risk of infection contamination by aerosol (Reicks, 2010). The use of off-site PRRSV acclimation facilities should preferably not increase the risk of exposure of other populations of pigs in the area to that PRRSV strain. With an off-site facility transport between that facility and the main unit can be another risk for some farms.

1.3.4.1.4 Acclimation Flow/Continuous versus All-In /All-Out: Some producers have used continuous flow acclimation units to maintain a source of PRRSV that is available for acclimation. The continued circulation and replication of PRRSV that is inherent with this type of pig flow increases the potential for genetic mutation of the PRRSV. At some point a PRRSV that is significantly different from the original breeding herd PRRSV isolate might arise and this “evolved” PRRSV may be introduced to the breeding herd. The existing breeding herd immunity to the original PRRSV may not provide adequate cross protection to this newly evolved PRRSV strain. Continuous flow is not the first choice for an acclimation facility as the health of the replacements is jeopardized and PRRS viral strain “drift” may be enhanced (Roberts, 2002). In the bigger picture, the continuous flow acclimation facility may be the only facility that is initially available. As stated earlier, all-in/all-out pig flow practices in the acclimation facility are recommended (Dee, 2004).

1.3.4.1.5 Strain monitoring: In PRRSV positive herds that have a circulating PRRSV strain, updating the exposure strain over time has been used in the field. The goal of this procedure is to attempt to maintain the immunity of the breeding herd to the changing PRRSV in that population. This approach requires routine re-isolation of the resident PRRSV, sequence comparison and then updates to the exposure program (Torrison et al, 2003). This approach is intended to assist in controlling the impact of PRRSV strain “drift” over time and does not result in PRRSV elimination from the herd.



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1.3.4.2. Breeding Herd Exposure

Field type PRRSV exposure of all sows and boars in the herd is used to ensure that all sows and boars have been exposed at a “single point in time” and that all become immune simultaneously (Corzo et al, 2010). This provides a greater opportunity for elimination of PRRSV from the breeding herd. In some situations, an adequate number of replacement gilts and boars can be purchased and then exposed to the herd specific PRRSV strain. The breeding herd is then closed for at least 180 days (Alfonso et al, 2005; Schaefer and Morrison, 2007; DuBois, 2007). In most cases, the period of closure will be longer (Torremorell et al, 2003; Schaefer and Morrison, 2007). A more commonly accepted period of herd closure is 200 days (Yeske, 2009).

Field type PRRSV exposure of pregnant sows will likely cause some sow deaths and/or reproductive problems with the risk of abortion, stillborn pigs and transplacental infection of piglets in the later stages of pregnancy (Bruner, 2007). Infection of piglets in the uterus of late pregnancy sows can increase the risk of PRRSV disease in suckling, nursery, and finisher pigs (Bruner, 2007).

1.3.4.3. Growing Herd Exposure

Field type PRRSV exposure of all growing pigs in a nursery or finisher is used to ensure that all growing pigs have been exposed at a “single point in time” and that all become immune simultaneously (Pittman, 2007). This provides an opportunity for more uniform immunity and therefore reduced shedding of PRRSV prior to the introduction of replacements to a continuous flow nursery or finisher. Intentional exposure to virulent field type PRRSV strains may not be an option due to significant production losses associated with clinical disease (Dufresne, 2003). Exposure to less virulent PRRSV strains may present an acceptable risk given that the elimination of the virus from the site is the long-term goal.

2. Management practices to reduce PRRSV challenge

Best management practices should be implemented prior to attempting PRRSV elimination. This would include internal and external biosecurity, sanitation and management procedures (Dufresne, 2003; Torremorell et al, 2000; Zimmerman, 2007; McCaw, 1995). Attention to detail in these areas will improve the odds of success.

The PRRS challenge reducing tools through best management practices lower the amount of PRRSV that is available in the environment to infect pigs in the population. The goal is to reduce the number of viral particles below the infectious level allowing the virus to die out on the farm.

2.1 McREBEL™ PRRS

McREBEL™ PRRS is an acronym for a PRRSV management strategy. McREBEL™ stands for **Management Changes to Reduce Exposure to Bacteria to Eliminate Losses** (McCaw, 1995). The program was developed and named by Dr. Monte McCaw at North Carolina State University. The primary goal of the program is to reduce secondary bacterial infections but it is also effective in reducing PRRSV transmission. The McREBEL™ PRRS program is a simple, low cost program to



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implement and is a practical strategy for minimizing nursery and farrowing room losses while the breeding herd is being stabilized.

The program can be challenging to implement where farm staff find it difficult to resist the temptation to foster after 24 hours, or if euthanizing of piglets presents a problem. The program requires a change of mind set to be successful.

The McREBEL™ PRRS program includes controls on the timing of crossfostering, movement of sows or piglets between rooms, use of nurse sows, piglet euthanasia, piglet processing, and nursery pig flow (McCaw, 1995).

The importance of sustained compliance to the McREBEL™ PRRS program cannot be overemphasized. It appears to be one of the most critical areas to achieve PRRSV elimination in a sow herd. McREBEL™ PRRS procedures should be adhered to until testing has confirmed successful PRRSV elimination (Polson et al, 2010). Recommended McREBEL™ PRRS Limited Crossfostering Production Procedures can be seen in Appendix 1 (McCaw, 2006).

2.2 Biosecurity

Internal biosecurity deals with the control of movement of virus from infected to non-infected animals within the same population. This discipline can be used very early in an acute PRRSV outbreak to maximize the number of non-infected weaned pigs. It is also used to reduce the presence of virus in the facility after a PRRS outbreak.

Internal biosecurity covers many of the same procedures as external biosecurity, but the focus is within the barn. The internal biosecurity tools can be used to reduce infection rates within a population. An example of this would be control of fomites or objects that can carry the PRRSV from pig to pig, such as needles, tooth nippers, hog snares, shovels, brooms, etc. Internal biosecurity covers pig flow issues such as stop-movement on sows and unidirectional pig flow. Complete biosecure change areas such as internal “Danish entries” may be established to control movement of PRRSV from infected to non-infected areas of a building. All-in-all-out pig flow is an essential part of internal biosecurity (Pitkin et al).

Key Internal Biosecurity Recommendations During a PRRSV Infection (Charbonneau, 2007)

- Stop flow of PRRS positive pigs to negative nurseries.
- Stop movement of sows during acute PRRSV outbreaks in breeding herds.
- Isolate aborting sows.
- Thoroughly clean crates or pens where sows have aborted.
- Change needles between sows.
- Change needles between litters.
- Change disposable gloves between litters.
- Clean boots and hands and change coveralls after working with sick pigs known to be shedding the virus.
- Use separate shovels, brooms or scrapers for manure passage and feed alley.



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- Discontinue pre-farrowing manure feedback.
- Apply lime or other dry disinfectants to hallways.
- Duplicate sets of piglet processing equipment to allow increased disinfectant contact time.
- Control feed cart traffic between rooms.
- Disinfect piglet processing carts or discontinue use of processing carts.
- Set up effective hospital and recovery pens in growing pig flows.
- Euthanize animals with poor prospect of recovery on a timely basis. Take these pigs to a remote area of the barn so that blood from the euthanasia process does not infect susceptible pigs.
- Maintain a unidirectional flow of pigs - no pigs moving back into younger pig populations or lingering around in sick pens. “Treat and recover or treat and euthanize!”
- Batch farrowing every 4 weeks will facilitate all-in/all-out management of the farrowing building (Bonneau, 2010).
- It can also be beneficial to stop breeding for 3 weeks (Bonneau, 2010).
- Empty, clean and disinfect refrigerators or freezers that maintain viable PRRSV.

External biosecurity deals with the control of entry of new pathogens in a herd. Several procedures have been described to prevent herd infection. With the demonstration of long-distance aerosol transmission of infectious PRRSV, air filtration has gained popularity as an effective external biosecurity procedure in pig dense areas (Otake et al, 2010; Reicks, 2010). Biosecurity is only as effective as the weakest link in the program and therefore air filters alone will not provide a comprehensive program unless external and peripheral biosecurity is also tight.

For a more in-depth reading on PRRS biosecurity procedures, the AASV PRRS Biosecurity Manual can be consulted at http://www.aasv.org/aasv/PRRSV_BiosecurityManual.pdf.

2.3 Sanitation

Sanitation is used between batches of pigs in order to completely eliminate the PRRSV from the targeted areas of the facility. All organic material, including feces, urine, feed, bedding and body fluids should be completely removed and the surfaces power washed. It is recommended that a detergent be used in the washing to ensure removal of biofilms.

Once clean, an efficacious disinfectant should be applied throughout the pen area. Some examples of products proven to be efficacious against PRRSV are quaternary ammonium+ glutaraldehyde mixtures and modified potassium monopersulfate. These products should be applied at a 0.8% and 1% concentration, respectively. All surfaces need to be thoroughly covered and a minimum contact time of 2 hours is required. In winter all areas to be disinfected should be heated to ensure the surface temperatures are above 10° C so that the disinfectants can be effective (Schneider, 2010).

Following cleaning, the facility must be allowed adequate downtime to allow drying time after disinfection. Allowing the room to dry is the most important step in the sanitation protocol for complete inactivation of the virus.

For a more in-depth reading on PRRS sanitation, the AASV PRRS Biosecurity Manual can be consulted at http://www.aasv.org/aasv/PRRSV_BiosecurityManual.pdf.



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PRRS Elimination Programs

1. Depopulation

1.1 Whole Herd Depopulation/Repopulation

This method involves the removal of all breeding and/or growing pigs from the farm, disinfecting the facilities and restocking the farm with PRRSV negative pigs (Corzo et al, 2010).

Depopulation/repopulation is used when a farm has a low probability of achieving profitability due to multiple disease problems and there are no other cost effective interventions available that have a reasonable probability of success. Herds with multiple strains of PRRSV and a significant number of other diseases are better candidates for depopulation/repopulation (Roberts, 2002; Corzo et al, 2010) A herd that is looking for a rapid genetic change may also use this method. However, this method should not be used before the veterinarian has found the source of PRRSV and assessed the risks factors that contributed to the PRRS outbreak otherwise the herd might break again with PRRS soon after repopulation (DeBuse, 2007). Moreover, it is essential that a reliable supply of naïve seronegative replacement animals be available after the repopulation (DeBuse, 2007; Hill et al, 2004).

Advantages	Disadvantages
<ul style="list-style-type: none"> - High degree of efficacy - Solves multiple disease problems at the same time - Can result in genetic improvements - Vast experience using the method in the veterinary industry 	<ul style="list-style-type: none"> - Costly - Requires multiple sites for off-site breeding of new clean stock and finishing out of infected pigs - Re-infection can occur during the repopulation process (or at any later point)

Note to reader: there is a good summary by Paul Yeske on the cost benefit of depopulation/repopulation as compared to herd closure methods (Yeske, 2010).

1.2 Farrowing Depopulation

Once the breeding herd has developed immunity to the farm specific PRRSV, the main reservoir for viral circulation in a farrow to isowean unit is the piglet. Emptying the farrowing facility will eliminate the piglet reservoir for viral circulation. It also provides an opportunity for effective implementation of sanitation programs. Timing the farrowing depopulation is essential and must not occur before the risk of “born viremic” piglets has been eliminated (Misener, 2010).

Advantages	Disadvantages
<ul style="list-style-type: none"> - Does not rely on the McREBEL™ PRRS program - Controls for human error 	<ul style="list-style-type: none"> - Lost production

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1.3 Nursery and/or Finisher Depopulation/Repopulation

Once there is a consistent source of PRRSV negative weaned pig or feeder pigs, the nursery or finisher can be depopulated and then repopulated with the PRRSV negative stock (Dee and Joo, 1997). This intervention requires vigorous cleaning and disinfection before the introduction of PRRSV negative pigs (Dufresne, 2003; Torremorell et al, 2003).

Advantages	Disadvantages
<ul style="list-style-type: none"> - High efficacy - Productivity gain from the one time building sanitation 	<ul style="list-style-type: none"> - Requires off-site nursery or temporary remodelling of finisher facility to accommodate young pigs or longer stay in the farrowing crates

1.4 Nursery or Finisher Partial Depopulation /Repopulation

In finishing barns that are all in/all out (AIAO) by section, PRRSV elimination can be achieved by partial depopulation, putting PRRS-negative finishers into emptied, cleaned and disinfected sections, while other sections still contain PRRS positive animals (Andreason, 2000). This intervention requires vigorous cleaning and disinfection before the introduction of PRRSV negative pigs in empty rooms (Dufresne, 2003; Torremorell et al, 2003). This technique will be more successful for strains that are less prone to spread via aerosol and lend themselves to be controlled by strict application of internal biosecurity techniques. Specific procedures need to be established between the PRRS positive and negative areas to control the risk of PRRSV movement. Failure due to aerosol spread is a distinct possibility (Charbonneau, 2010).

Advantages	Disadvantages
<ul style="list-style-type: none"> - Good efficacy but lower than total depopulation (80%) (Andreason, 2000) - More convenient than total depopulation 	<ul style="list-style-type: none"> - Higher risk of re-infection than total depopulation

2. Test and Removal (TR)

Test and Removal is based on serological/virological testing of the breeding herd and then subsequently culling seropositive/infected animals (Dee et al, 2001; Corzo et al, 2010). The sow herd is monitored, and when less than 15% of animals are PRRS antibody test positive, the test-remove option is implemented (Dee et al, 2000). The entire herd is tested at the same time using both the PRRS ELISA and PCR tests. Any sows that are positive to either test are immediately culled (Dee et al, 2001; Roberts, 2002). If PRRSV infection was detected post-weaning, infected nursery and/or finishing facilities should be depopulated 24-48 hours prior to Test and Removal (Dee et al, 2001). Following completion of the Test and Removal, the breeding herd, nursery and finisher pigs (if applicable) are monitored monthly by ELISA for 12 consecutive months. The sample size should be capable of detecting at least 1 positive pig at an estimated prevalence of \geq



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5% at 95% confidence for the sow herd and at least 1 positive pig at an estimated prevalence of $\geq 30\%$ at 95% confidence for the nursery/finisher pigs (Dee et al, 2001). The probability of success increases with the time elapsed. Current recommendations are to wait for twelve months before considering the herd negative. PRRS naive, seronegative replacement stock can act as sentinels and be monitored more intensively.

Advantages	Disadvantages
<ul style="list-style-type: none"> - High degree of efficacy (100%) (Dee et al, 2001) - Lower risk than Wean and Removal (Dee et al, 2000) 	<ul style="list-style-type: none"> - Testing costs can be high - Labor intensive - Cost of premature removal of infected or seropositive breeding animals - Feasible only in herds with a low (<15%) seroprevalence in the breeding herd

3. Wean and Removal (WR)

Wean and Removal is similar to the Test and Removal program but is specifically focused on weaned sow groups. Sow groups are tested prior to farrowing with PRRS ELISA and PCR (Sandri, 2001). Normally there will be very few PCR positive animals and these are removed immediately without farrowing. PRRS ELISA positive sows are culled at weaning and replaced with PRRSV free replacements. A Wean and Removal program normally proceeds for 20 weeks (Roberts, 2002), at minimum, all sows should go through a testing phase at least once. It is slow in comparison to the immediate Test and Removal program. There is a risk that sows that were PRRS ELISA negative prior to farrowing may be infected by one of the as yet undetected PRRS PCR positive sows in the herd. The PRRS PCR negative / PRRS ELISA positive sows that are allowed to farrow may intermittently shed virus for up to 90 days after infection and be a source of infection for the PRRS ELISA negative sows.

Advantages	Disadvantages
<ul style="list-style-type: none"> - More practical for large herds than TR - Less expensive than TR because seropositive pregnant sows are not culled 	<ul style="list-style-type: none"> - Less effective than TR

4. Production of PRRSV Negative Pigs from Positive Sows

The goal of this strategy is to produce PRRSV negative animals from a PRRSV positive herd in a manner to populate a newly established herd (Torremorell et al, 2000). This program involves 4 steps:

- Identification of the donor population: A population of seropositive animals where no PRRSV is circulating is identified.



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- Breeding and gestation of donor population

- **Farrowing and weaning:** Farrowing should happen in an isolated area. Weaning should happen as early as possible around 5 to 7 days of age. Weaned pigs should be moved as soon as possible to an off-site nursery to avoid any cross-contamination.

- **Off-site nursery stage and testing:** The nursery site should be operated on an all-in/all-out basis. At least one sentinel pig should be added per pen and monitored periodically. The pigs should remain together for a minimum of 12 weeks to allow for maternal antibody depletion. At that time, all the principals should be tested with ELISA. If both sentinels and principals have remained seronegative, then the population is considered negative (Torremorell et al, 2000).

Advantages	Disadvantages
<ul style="list-style-type: none"> - Preserves genetics - Can improve overall health status and performance of offspring - Fair efficacy (71%) (Torremorell et al, 2002) 	<ul style="list-style-type: none"> - Transmission of PRRSV from sow to offspring can result in the production of infected batches of weaners

5. Herd Closure and Rollover

Herd closure and rollover has become the most widely used method for eliminating PRRSV from sow herds (Corzo et al, 2010). This method consists of interrupting the introduction of incoming replacement females and males into the breeding herd for at least 6 months plus the elimination of seropositive animals over time. Herd closure brings an overall improvement in health and productivity (DuBois, 2007). The process of implementing PRRSV elimination by herd closure involves the following steps:

5.1 Herd expansion

Herd expansion involves the introduction of staggered ages of replacement animals prior to closure of the breeding herd. In this way no new PRRSV naïve animals need to be introduced to the breeding herd for 8 months after closure. This large batch of breeding stock replacements will be exposed at the same time as the breeding herd on a single exposure date (DeBuse, 2007). An alternative approach to maintaining breeding targets that does not involve exposure of the replacements gilts is to use of an off-farm breeding facility that is filled with naïve seronegative gilts. This might be a good alternative approach if not enough space is available in the breeding herd to allow for loading of the herd (Torremorell et al, 2003). If the herd closure is not successful another location must be found for the naïve gilts to farrow as entry of pregnant naïve gilts will lead to reproductive problems in the sow herd and increased respiratory disease in the downstream growing pigs (Charbonneau, 2010).

Planned Live Virus exposure

Planned Live Virus exposure of the breeding herd with homologous virus or commercial MLV PRRSV vaccines occurs once the herd has been closed. Live virus exposure increases the uniformity of herd immunity and eliminates any sub-populations of previously PRRSV non-exposed animals. The objective of exposing all animals at the same time is to ensure that the entire population is exposed and has developed an effective immune response. Without live virus



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exposure subsequent spread and infection of negative subpopulations will increase the period of time required for shedding to stop. The increased shedding period is due to sporadic spread of virus throughout the herd. Although persistently infected animals may exist temporarily, if there are no susceptible animals remaining in the herd, the ability of the virus to circulate within herd will be significantly reduced or eliminated (Corzo et al, 2010).

5.2 Introduction of naïve seronegative replacement animals

Before the introduction of naïve seronegative replacement gilts, sentinel animals should be commingled with seropositive sows and gilts in a separate facility to determine whether virus is still being shed (Torremorell et al, 2003). After the introduction of naïve seronegative replacement animals, careful measures should be taken to segregate the naïve seronegative replacement gilts from the replacement gilts that entered immediately prior to planned exposure and herd closure as these are the animals most likely to remain infected (Torremorell et al, 2003).

5.3 Culling of seropositive females

Once the first naïve seronegative replacement gilts are introduced into the herd the previously PRRSV exposed females will be removed through the normal culling process in most commercial herds. An accelerated culling program of seropositive sows can be used where the goal is to proceed to PRRS naïve serologic status as quickly as possible (Dufresne, 2003).

5.4 PRRSV elimination from the growing pigs

PRRSV elimination in the growing pig flow should be performed when there is a high level of confidence that the pig flow will remain negative. This step requires depopulation of the nursery followed by vigorous cleaning and disinfection prior to repopulation with PRRSV negative pigs (Dufresne, 2003; Torremorell et al, 2003).

5.5 Post-Elimination Monitoring

Throughout the process, routine serologic monitoring is required: sentinels before the introduction of naïve seronegative replacement animals, naïve seronegative replacement animals and growing pigs. Monitoring in the production flow should be performed on a monthly basis and with adequate statistical power to detect infection if present (Torremorell et al, 2003).

Advantages	Disadvantages
<ul style="list-style-type: none"> - High degree of efficacy (91-100%) (Dee et al, 2001; Dubois, 2007) - Less labor intensive than TR or WR - Does not require excessive removal of breeding animals - Less expensive than depopulation, TR or WR 	<ul style="list-style-type: none"> - Might require off-site breeding facilities - Requires a long time to complete



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Monitoring Tools

Testing to Establish a Successful PRRSV Elimination

The ability to eliminate PRRSV from a herd has been clearly demonstrated. However, one area that has not been well defined is herd testing to verify successful PRRSV elimination. Low prevalence of PRRSV in the herd makes accurate testing and validation of negative PRRSV status challenging (Morrison, 2009).

1. Tests:

1.1 ELISA

An early study reported 96.6% sensitivity and 100% specificity for PRRS ELISA (Cho et al, 1996), in a truly naïve population. However variations can be found in the field depending on farm factors and the age of animal sampled. Singleton reactors (one positive sample in a batch of negative animals) should be confirmed as negative by a different antibody assay such as the indirect fluorescent antibody test (IFAT) and PRRS PCR should be conducted to eliminate the possibility of very recent infection (Charbonneau, 2010). Another confirmation of PRRSV negative status can be obtained by resampling the animal and pen mates at a further date (at minimum 10 days later) as this confirms that neither seroconversion nor spread to other animals has occurred.

Complicating interpretation of serum antibody tests are antibodies remaining from an earlier PRRSV infection prior to the elimination program or from maternal antibodies from the mother. Sampling should target animals where the likelihood of finding these antibodies is low. These reactors are not false positive in the sense that the test detected real antibodies, but they do not reflect recent or current infections. Recommendations to minimize these positive readings are to sample growing animals as old as possible in the production system to let maternal immunity decline, and sample sentinel animals where possible.

It is also important to realize that the IFAT and PRRS PCR are strain specific.

1.2 PCR

It is important to keep in mind that estimating herd PRRSV prevalence is extremely important in designing PCR testing programs that will maintain high levels of sensitivity and specificity. A negative PCR test is not a guarantee that an individual animal is not infective.

1.2.1 Serum

Although several different PCR tests exist, all have high sensitivity and specificity. For PRRSV real-time RT-PCR individual testing, a sensitivity of 95.5% was reported while a sensitivity of 100% was reported for the SYBR Green RT-PCR (Martinez et al, 2008). Although an early study reported a specificity of 100% for PRRSV RT-PCR test, a subsequent study reported a specificity of 96.4% for RT-nPCR (Suarez et al, 1994; Wagstrom et al, 2000). Therefore both false positive and false negative results exist with PRRSV PCR-based assays and results may vary between laboratories (Truyen et al, 2006; Zimmerman, 2008). It is a common practice to pool individual serum samples for laboratory submission because PCR tests are quite expensive. This practice

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decreases the pool level sensitivity to 84.5% for pools of 5:1 and to 82.0% for pools of 10:1 (Carmichael et al, 2010). The pool level specificity for pools of 5:1 and 10:1 was reported as 99.0% and 97.7%, respectively. However, when proper herd sampling protocols are used, the herd level sensitivity and specificity can be 100% for both 5:1 and 10:1 pools (Carmichael et al, 2010).

1.2.2 Oral fluids

A new protocol for PRRS surveillance was recently described (Zimmerman et al, 2007; Weeks et al, 2010). Oral fluids are collected by allowing the pigs to chew on a cotton collection rope that is placed in each pen for approximately 20 minutes. The oral fluids are then harvested and tested by PCR. The results suggest that oral fluid sampling for PRRSV could be a practical substitute for blood sampling.

2. Use of Sentinels

Naïve seronegative animals are used in PRRSV elimination programs for testing for the presence of circulating PRRSV. Sentinel animals should be commingled with seropositive sows and gilts in a separate facility to determine whether virus is still being shed (Torremorell et al, 2003). Sentinels can also be commingled with the off-spring of positive sows to assure that the off-spring remains negative. In this case, sentinels are mixed with weaned pigs (Torremorell et al, 2000). Sentinels should be distributed evenly within the seropositive population at the rate of one sentinel per pen for nursery pigs (Torremorell et al, 2002). In some protocols, sentinels are tested serologically before the introduction in the herd and then every month thereafter, with final testing not less than 4 weeks after removal from in-contact animals (Torremorell et al, 2000). In other protocols, sentinels are kept inside the units for 2 months and tested on a weekly basis until removal (Alfonso et al, 2005). Sentinels should only be placed into the herd after a high level of confidence has been achieved that the PRRSV is no longer present in the population.

3. Monitoring Protocols for PRRSV Elimination

3.1 Animal selection:

To better detect PRRSV circulation, animals with the higher risk of infection can be selected: sentinels, naïve replacement, culled gilts and sows, sick pigs, weaned pigs (when using PRRSV PCR test), pigs towards the end of nursery or grower-finisher (when using PRRSV ELISA test) (Morrison, 2009; Torremorell et al, 2002; Torremorell et al, 2003; Desrosiers and Boutin, 2002, Batista et al, 2002; Ruen, 2003).

3.2 Sampling size:

The sampling size required to detect PRRSV virus circulation is dependant on the targeted confidence level, the sensitivity and specificity of the test used and the minimum prevalence rate that is selected.

As an example for a PRRSV elimination monitoring protocol, Alfonso et al reported following a statistical sampling plan directed at detecting at least one positive animal given a prevalence ranging between 5 to 10% prevalence with a 95% confidence interval (Alfonso et al, 2005).



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As prevalence decreases within a herd, the sampling size required to detect virus circulation at a 95% confidence level increases (Morrison, 2009). Also, as prevalence decreases within a herd and the sampling size increases, the percentage of false positive reactions increases. Testing in series is the best approach to detect and confirm positive animals in low prevalence herds (Morrison, 2009). To determine sample size for random sampling, statistical tables (Appendix 3) are available (Morrison, 2009; Ramirez, 2008).



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Appendix 1: Recommended McREBEL Limited Crossfostering Production Procedures (McCaw, 2006)

1. Don't crossfoster piglets after 24 hours of age
 - a. Move the minimum number of pigs necessary to load functional teats
 - b. Don't crossfoster to create uniform size or sex litters
 - c. When EXTRA medium or large pigs must be moved, do match them by size and milking ability of litter and receiving sows
 - d. Ensure smallest piglets are given lowest priority for functional teat assignment, leave on birth sow or move as "extras" when more piglets than available teats

MAXIMIZE THE NUMBER OF PIGLETS REMAINING ON THEIR BIRTH MOTHER!
Otherwise, maximize the number of piglets remaining on the colostrum mother

2. Don't move piglets between rooms
 - a. Follow strict All In – All Out production

THE LITTER IS NOW THE ALL IN-ALL OUT UNIT!

3. Remove very sick, moribund or bad body condition pigs from the system
 - a. Sell or eliminate piglets at weaning that are too light to survive in the nursery and have poor body condition
 - b. Eliminate immediately piglets that don't quickly get better after treatment
 - c. Eliminate very thin, starve-out, lame, light body weight, long-haired, chronically sick piglets as they are found

A PIGLET HELD-BACK FROM WEANING TAKES A TEAT AWAY FROM A YOUNGER POTENTIALLY HEALTHIER PIG!

4. Nursery care practices to maximize piglet survival and performance
 - a. Size piglets into pens carefully
 - b. Place smallest in warm, non-drafty part of room
 - c. Hand feed smallest piglets 4 times a day for 5 days
 - d. Switch rations based upon weight of pen, not room
 - e. Use heat lamps and/or plastic lying pads for small piglets
 - f. Lower one nipple/pen and jam it open for the first 24 hours to help piglets find water

DON'T EXPECT TO WEAN ANY MORE QUALITY PIGLETS THAN THERE ARE FUNCTIONAL TEATS IN A FARROWING ROOM.

TO MAXIMIZE THE NUMBER OF PIGLETS WEANED PER ROOM, MAXIMIZE THE NUMBER OF FUNCTIONAL TEATS BY PROPER GILT SELECTION AND SOW CULLING.



PRRS Control and Elimination Tool Kit

Appendix 2: Post-Infection PRRSV Timeline (McCaw, 2006)

The following timeline lists the various events that occur in the clearance of PRRSV from an individual pig. In some pigs, it can take a long time to develop the full level of immunity that is required to clear virus from the body.

Days Post-infection

- 10 – 30 Viremia (virus can be isolated from the blood), strong PRRSV ELISA antibody response
- 20 – 30 Earliest time neutralizing antibody can be detected in blood
- 60+ Full or peak titer of neutralizing antibody in blood reached
- 100 – 150 Tonsil/lymph nodes become PRRSV negative (for pigs infected in the nursery), many pigs become PRRSV ELISA negative (<0.4 S/P ratio), still Serum Neutralizing positive
- 150++ Tonsil/lymph nodes become PRRSV negative (for pigs infected *in utero*)
- 200 Duration of herd-closure needed post-outbreak to eliminate PRRSV from the herd

Appendix 3. Statistical sampling size table taken from Morrison, 2009.

Table 1: Sample size required to detect at least 1 positive case with 95% confidence with varying population size and prevalence in the underlying population (Cannon & Roe).

Pop size	50%	40%	30%	25%	20%	15%	10%	5%	2%	1%	1%	0%
10	4	5	6	7	8	10	10	10	10	10	10	10
20	4	6	7	9	10	12	16	19	20	20	20	20
30	4	6	8	9	11	14	19	26	30	30	30	30
40	5	6	8	10	12	15	21	31	40	40	40	40
50	5	6	8	10	12	16	22	35	48	50	50	50
60	5	6	8	10	12	16	23	38	55	60	60	60
70	5	6	8	10	12	17	24	40	62	70	70	70
80	5	6	8	10	13	17	24	42	68	79	80	80
90	5	6	8	10	13	17	25	43	73	87	90	90
100	5	6	9	10	13	17	25	45	78	96	100	100
120	5	6	9	10	13	18	26	47	86	111	120	120
140	5	6	9	11	13	18	26	48	92	124	139	140
160	5	6	9	11	13	18	27	49	97	136	157	160
180	5	6	9	11	13	18	27	50	101	146	174	180
200	5	6	9	11	13	18	27	51	105	155	190	200
250	5	6	9	11	14	18	27	53	112	175	228	250
300	5	6	9	11	14	18	28	54	117	189	260	300
350	5	6	9	11	14	18	28	54	121	201	287	350
400	5	6	9	11	14	19	28	55	124	211	311	400
450	5	6	9	11	14	19	28	55	127	218	331	450
500	5	6	9	11	14	19	28	56	129	225	349	500
600	5	6	9	11	14	19	28	56	132	235	379	597
700	5	6	9	11	14	19	28	57	134	243	402	691
800	5	6	9	11	14	19	28	57	136	249	421	782
900	5	6	9	11	14	19	28	57	137	254	437	868
1000	5	6	9	11	14	19	29	57	138	258	450	950
1200	5	6	9	11	14	19	29	57	140	264	471	1102
1400	5	6	9	11	14	19	29	58	141	269	487	1236
1600	5	6	9	11	14	19	29	58	142	272	499	1354
1800	5	6	9	11	14	19	29	58	143	275	509	1459
2000	5	6	9	11	14	19	29	58	143	277	517	1553
3000	5	6	9	11	14	19	29	58	145	284	542	1895
4000	5	6	9	11	14	19	29	59	146	286	556	2108
5000	5	6	9	11	14	19	29	59	147	290	564	2253
6000	5	6	9	11	14	19	29	59	147	291	569	2358
7000	5	6	9	11	14	19	29	59	147	292	573	2437
8000	5	6	9	11	14	19	29	59	147	293	576	2498
9000	5	6	9	11	14	19	29	59	148	294	579	2548
10000	5	6	9	11	14	19	29	59	148	294	581	2588