PEER REVIEWED

COMMENTARY

Proposed modifications to porcine reproductive and respiratory syndrome virus herd classification

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Summary

A standardized system for classifying the porcine reproductive and respiratory syndrome virus (PRRSV) status of swine herds is necessary for communication between veterinarians and producers. The 2011 classification system has been widely adopted by producers and veterinarians worldwide. In 2018, a working group met to revisit the system and make recommendations for changes. The most significant modification was to the classification of positive unstable and positive stable breeding herds. Recommended diagnostic protocols for promotion of herds to each status were modified and recommended diagnostic protocols to maintain a status were added. The growing pig classification for PRRSV was also modified.

Keywords: swine, porcine reproductive and respiratory syndrome virus, herd classification, disease status, modification

Received: June 11, 2020 Accepted: February 3, 2021

Resumen - Modificaciones propuestas a la clasificación del virus del síndrome reproductivo y respiratorio del cerdo

Es necesario un sistema estandarizado para clasificar el estatus del virus del síndrome respiratorio y reproductivo porcino (PRRSV) en las piaras de cerdos para la comunicación entre veterinarios y productores. El sistema de clasificación de 2011 ha sido ampliamente adoptado por productores y veterinarios de todo el mundo. En 2018, un grupo de trabajo se reunió para revisar el sistema y hacer recomendaciones para cambios. La modificación más significativa fue la clasificación de las piaras de reproductores positivas inestables y positivas estables. Se modificaron los protocolos de diagnóstico recomendados para la promoción de piaras a cada estatus y se agregaron protocolos de diagnóstico recomendados para mantener un estatus. También se modificó la clasificación del PRRSV para los cerdos en crecimiento.

Résumé - Modifications proposées à la classification des troupeaux en lien avec le virus du syndrome reproducteur et respiratoire porcin

Un système normalisé de classification du statut des troupeaux de porcs relativement au virus du syndrome reproducteur et respiratoire porcin (VSRRP) est nécessaire pour la communication entre les vétérinaires et les producteurs. Le système de classification de 2011 a été largement adopté par les producteurs et les vétérinaires du monde entier. En 2018, un groupe de travail s'est réuni pour revoir le système et faire des recommandations de changements. La modification la plus significative concernait la classification des troupeaux reproducteurs positifs instables et positifs stables. Les protocoles de diagnostic recommandés pour la promotion des troupeaux à chaque statut ont été modifiés et les protocoles de diagnostic recommandés pour maintenir un statut ont été ajoutés. La classification des porcs en croissance pour le VSRRP a également été modifiée.

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This article is available online at http://www.aasv.org/shap.html.

Holtkamp D, Torremorell M, Corzo CA, Linhares DCL, Almeida MN, Yeske P, Polson DD, Becton L, Snelson H, Donovan T, Pittman J, Johnson C, Vilalta C, Silva GS, Sanhueza J. Proposed modifications to porcine reproductive and respiratory syndrome virus herd classification. *J Swine Health Prod*. 2021;29(5):261-270.

I n 2009, a committee met to discuss constructing terminology to classify swine herds according to porcine reproductive and respiratory syndrome virus (PRRSV) status. Their work culminated in a peer-reviewed paper titled "Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status" published in the *Journal of Swine Health and Production.*¹ Before publication, the classification system was reviewed and approved by the board of directors of the American Association of Swine Veterinarians (AASV).

The classification system developed consisted of four categories for breeding herds: Positive Unstable (I), Positive Stable (II), Provisional Negative (III), and Negative (IV). Category II was further subdivided into II-A for herds not undergoing elimination and II-B for herds undergoing elimination. The system was built using two criteria: virus shedding and previous exposure to the virus. The supporting evidence for a herd to be promoted to each category was based solely on objective diagnostic results. Expected clinical signs and other subjective information for each category were noted but not included in the supporting evidence. Supporting evidence needed to maintain a herd in a category, after it had been promoted to that category, was not delineated. The most contentious debate regarding the original classification system centered on the definition of stable and the supporting evidence for the promotion of a breeding herd to the Positive Stable (II-A or II-B) category. The committee defined the term "stable" as a breeding herd with sustained and confirmable lack of detectable viremia in weaning-age pigs and promotion to Positive Stable (II-A or II-B) was based on testing serum for PRRSV by reverse transcriptase-polymerase chain reaction (RT-PCR). For supporting evidence, the committee recommended testing 6 pools of 5 serum samples from 30 weaning-age pigs monthly for 4 consecutive months with no positive results. The 30 samples needed each time the herd was tested was based on the number of samples required to detect an expected prevalence of 10% with 95% confidence for any population size greater than 1000, assuming a diagnostic test sensitivity greater than 95% and random sampling from a population with a homogenous distribution of positive animals.² The committee considered the tradeoff between the cost and inconvenience of testing and

the confidence and ability to detect a low prevalence. A larger sample size with more frequent testing would have been preferred to detect a lower prevalence and increase the confidence level, however, the cost and inconvenience would limit adoption of the classification system.

The value of the system to classify the PRRSV status of swine herds is evident in how the 2011 system¹ has been used. Producers and veterinarians have used the classification system as a road map for managing PRRSV. It has facilitated communications between producers and veterinarians about health status, treatment and vaccination recommendations, and management of replacement animal introductions. The classification system has also been used to better manage biosecurity, including the establishment of down times, strategic placement of pigs, and strategic scheduling of pig movements, feed deliveries, and other activities. Having a standard classification has also provided researchers with a valuable tool with which to conduct research. Since it was published in 2011, the article summarizing the classification system has been cited over 100 times by researchers.³ As an example, it was used in a study published in 2012 to estimate the annual cost of PRRSV in the United States.⁴ The classification system has also facilitated PRRSV monitoring efforts to determine PRRSV infection status in US pig herds. Since 2011, the Morrison Swine Health Monitoring Project⁵ has used the classification system to monitor and report the incidence of PRRSV outbreaks and the proportion of swine breeding herds by PRRSV status in the United States. Finally, the classification system has been used to set premiums and discounts for weaned pigs according to the PRRSV status of the source sow farm (P. E. Yeske, DVM, meeting notes, 2020). The benefit of the classification system, when used for the purpose of setting premiums and discounts, arises from better pricing signals to more accurately set a price that reflects the real value of the pigs, and to incentivize the production of pigs that are negative for PRRSV.

Following the publication of the original classification system, several developments have led to calls for modifications to the system. For example, challenges with consistently weaning groups of pigs that are truly negative for PRRSV from breeding herds classified as Positive Stable (II), have led some to question the criteria and supporting evidence for those herds as some may have been falsely classified as stable. The evolution of new PRRSV isolates in the United States and other countries that, when present, make it more challenging to stabilize sow farms may have contributed to the challenge of consistently weaning groups of pigs that are truly negative for PRRSV. Development of new diagnostic sample types, such as oral fluids and processing fluids, and new diagnostic tests have presented opportunities to establish the status of herds more accurately and at a lower cost with less effort.

Objectives

Because of these new developments and the lessons learned from adoption of the original classification system, the AASV PRRS Task Force Committee voted to revisit the classification guidelines at the 49th AASV Annual Meeting in March of 2018. A working group, composed of the authors of this publication, was formed to propose modifications to the PRRSV classification system.

Methods

The working group met twice to discuss changes to the 2011 classification system.¹ The first working group meeting took place in Saint Paul, Minnesota at the University of Minnesota College of Veterinary Medicine on January 24 and 25, 2019. A summary of the first meeting was presented to the AASV PRRS Task Force Committee at the 50th AASV Annual Meeting in Orlando, Florida on March 9, 2019, and input from the committee was obtained. A second and final working group meeting was held in Ames, Iowa at Iowa State University College of Veterinary Medicine on June 5, 2019. The working group was made up of representatives from the swine industry including veterinarians from private practice, production systems, and industry, academia, and representatives from AASV and the National Pork Board. The modifications to the PRRSV classification system described in this publication were reviewed and approved by the board of directors of the AASV in the fall of 2019.

Consensus on modifications

The working group, with input from the AASV PRRS Task Force Committee, reached a consensus on the following proposed changes.

Category modifications for breeding herds

The Positive Unstable (I) category is split into two categories, representing high and low PRRSV prevalence, respectively. Category I-A represents positive unstable herds with a relatively high prevalence of pigs that are positive for PRRSV at weaning. Herds with unknown PRRSV status are classified as Category I-A by default. Category I-B represents positive unstable herds with a relatively low prevalence of pigs that are positive for PRRSV at weaning, characterized by intermittent detection of PRRSV in samples collected from suckling pigs, defined as piglets of any age from birth to weaning.

The Positive Stable (II) category will still represent herds that have achieved stability from PRRSV infection. The definition of stability is unchanged and includes herds with sustained and confirmable lack of detectable viremia in weaning-age pigs (ie, pigs within seven days of weaning), regardless of weaning age. The previous subcategories of Category II, Positive Stable Not Undergoing Elimination (II-A) and Positive Stable Undergoing Elimination (II-B), will no longer be used. Instead, Category II-vx is used to delineate positive stable herds where replacement animals, sows, and piglets may be immunized with a modified-live virus vaccine. Herds in which PRRSV-naive gilts are intentionally acclimated with live-virus inoculation could be included in II-vx as long as the criteria and supporting diagnostic evidence from the breeding herd in which they are introduced are met. Acceptable diagnostic samples to produce the supporting evidence include blood or other bodily fluids from suckling pigs. Processing fluids may be used to support the promotion and maintenance of a herd into this category, but it is not sufficient evidence alone. The diagnostic recommendations for promotion to this category were made more stringent to increase confidence that true herd stability has been achieved. The working group agreed that stable must be more explicitly defined to provide uniformity and ease of communication throughout the industry. The Provisional Negative (III) and Negative (IV) categories remain unchanged from the original 2011 paper.¹

Modification to supporting evidence required to move into a category

The working group stipulated that any modifications to the classification system must be practical, affordable, reliable, and straightforward to be adopted. However, there is generally a tradeoff between the cost and sensitivity of testing protocols. This tradeoff factored heavily in the final recommended modifications. With the addition of a second Positive Unstable category (I-B), the working group strengthened the supporting evidence required to promote a herd into the Positive Stable (II or II-vx) categories by increasing the number of serum samples tested from weaning-age pigs. This change was made to increase the likelihood of detecting positive pigs in herds with low prevalence and reduce the likelihood of falsely classifying herds as stable. For supporting evidence to promote a herd into the Positive Stable (II or II-vx) category, the working group recommended testing 6 pools of 10 serum samples from 60 weaning-age pigs by RT-PCR monthly for 4 consecutive months with no positive results. The number of samples doubled from the 30 samples recommended in the original classification system. The size of the pools tested also doubled from 5 to 10 serum samples per pool leaving the number of tests needed unchanged and, therefore, potentially reducing the diagnostic sensitivity of detecting individual positive pigs in the larger sample of 60 pigs from which sera were collected. The 60 pig sample size was based on the number of samples required to detect an expected prevalence of 5% with 95% confidence for any population size greater than 1000 assuming a diagnostic test sensitivity greater than 95% and random sampling from a population with an homogenous distribution of positive animals.²

In addition, the use of alternative population-based sample types to screen herds for PRRSV was incorporated. The working group viewed testing alternative sampling types as an easier, lower cost means to provide additional supporting evidence to increase the confidence of detecting positive pigs in the population. They include processing fluids,⁶⁻⁹ family oral fluids,¹⁰ udder wipes,

and environmental sampling.¹¹ One advantage of these new sample types is that they enable relatively easy and inexpensive sampling of more pigs, which lowers the cost of diagnostic testing per pig sampled. Testing more pigs more frequently may increase the sensitivity of the herd monitoring program, leading to a lower probability of falsely classifying a herd as stable. A recent report documented the increased use of processing and oral fluids for PRRSV diagnostics in the United States.⁹ The working group considered whether to recommend incorporating these new sample types and sampling schedules into the supporting evidence required to move into or remain in a category. Processing fluids, and family oral fluids in a limited way, were incorporated as alternatives sample types to serum. Environmental samples and udder wipes were deemed not sufficiently validated or lacking sensitivity, specificity, or both and were not included.

Testing piglet processing fluids by RT-PCR for PRRSV has become a useful screening tool to assess viral shedding in the breeding herd.⁶⁻⁹ It is an easy sample to collect, and a large number of pigs can be tested at a relatively low cost. Consequently, the working group included testing of processing fluids as a means to supplement serum testing from weaning-age pigs whenever possible. However, because pigs may become infected with PRRSV between processing and weaning, testing piglet processing fluids within the first week of age for PRRSV is insufficient to assess the shedding status of piglets at weaning, and therefore, cannot stand alone as diagnostic evidence to establish the shedding status of these pigs.

The use of family oral fluids is another sample type that can be used as supporting evidence to maintain a herd in a category and can be used to test a large number of animals at relatively low cost.¹⁰ However, success in collecting family oral fluids across systems can be variable, which limits the reliability of its use. Consequently, the working group included family oral fluids testing to be used as supporting evidence recommended to maintain a herd in a category.

Proposed new PRRSV herd classification

The description of each category provided here is a general characterization of a typical herd in each category.

Category I-A: Positive Unstable, High Prevalence

Herds that do not meet the criteria for any other category (I-B through IV) or do not have supporting diagnostic evidence are in the Positive Unstable, High Prevalence (I-A) category by default. Herds that have recently weathered an outbreak or herds where viral shedding and infection rates remain persistently high in the suckling piglet population will be in Category I-A. Clinical signs suggestive of PRRSV infections, including increased abortions, off-feed sows, stillborns, mummies, and preweaning mortality, are likely present. A large percentage of the breeding herd and suckling pigs are positive for antibodies to PRRSV and positive for PRRSV RNA by RT-PCR in serum, processing fluids, oral fluids, or all sample types. Replacement animals, sows, and piglets within this category may or may not be immunized with wild-type virus, modified-live virus vaccine, or inactivated PRRSV vaccine.

Category I-B: Positive Unstable, Low Prevalence

After 90 days of diagnostic testing to demonstrate a low prevalence of PRRSV infection in weaning-age pigs, a herd may be promoted to the Positive Unstable, Low Prevalence (I-B) category. The detection of PRRSV RNA by RT-PCR in serum from weaning-age pigs is intermittent, indicating low levels of viral shedding and transmission. The supporting diagnostic evidence for a herd to be promoted to Category I-B is in Table 1. Detection of PRRSV in the piglet population may be demonstrated with alternative sample types, including processing fluids. Few, if any, replacement breeding animals or sows will be positive for PRRSV by RT-PCR, and antibodies to PRRSV may be detected in all age categories of animals within the herd. Testing of diagnostic samples from the replacement breeding female and sow populations is not required as supporting evidence to promote a herd to Category I-B. Breeding replacement animals, sows, and piglets may or may not be immunized with a modified-live virus or inactivated vaccine. If a sample from a herd vaccinated with a modified-live virus

vaccine tests positive for PRRSV by RT-PCR, other validated molecular diagnostic methods, such as open reading frame 5 (ORF-5) sequencing, whole-genome sequencing, or PCR clamping assays, ^12 $\,$ may be used to distinguish whether positive RT-PCR results were due to wild-type virus or vaccine-like virus. If the ORF-5 sequence or other molecular diagnostic results indicates that the PRRSV isolate is vaccine-like, the result is considered negative for the purpose of changing categories. Deliberate exposure to wildtype PRRSV (ie, live virus inoculation) may be used for acclimation of replacement animals and resident sows but is not used on piglets.

Most commonly, herds in Category I-B exhibit mild or no clinical signs of PRRSV infection and have returned to near baseline levels of productivity as measured by pigs weaned per sow, number of pigs born, born alive, and farrowing rates. In herds where the goal is to control PRRSV, and where achieving stability is not considered feasible, Category I-B may be the target herd status. In herds where the goal is to control PRRSV and where achieving stability is considered feasible, Category I-B may be a transitional status to attaining stability with (Category II-vx) or without (Category II) the use of a vaccine to maintain some level of immunity against PRRSV in the herd. When PRRSV elimination is the goal, Category I-B is a transitional category for herds that are eliminating the virus by herd closure and rollover with Category IV as the target herd status.

Category II: Positive Stable

After 90 days of diagnostic testing to demonstrate a sustained lack of viremia in pigs at weaning, a herd may be promoted to Category II. The defining characteristic of herds in this category is producing weaning-age pigs that are consistently negative for PRRSV. This requirement must be supported by a consistent lack of detection in serum from weaning-age pigs tested for PRRSV RNA by RT-PCR (Table 1). In the new classification, the supporting evidence to promote a herd to Category II was elevated by recommending testing monthly serum samples from 60 weaning-age piglets by RT-PCR in pools of 10 instead of 30 samples tested in pools of 5. The larger 60 pig sample size is sufficient to detect a positive animal in a population with at least a 5% prevalence and 95% confidence.² However, testing in pools

of 10 may result in some reduction in the diagnostic sensitivity¹³ which may offset some of the benefit of testing more animals. The committee that developed the original classification system and the working group that proposed the modifications described in this paper both recognized that when a herd is transitioning to a Positive Stable (Category II) status, the expected prevalence of positive animals will be very low. In those cases, a balance between the cost, the inconvenience of sampling, and the increased confidence of detecting a very low prevalence was sought. In the new classification system, the addition of populationbased testing of processing fluids or other sample types as they become available, such as family oral fluids or udder wipes, may be used to provide additional evidence to support the PRRSV negative status of the pigs at weaning, but they cannot stand alone to promote or maintain a herd in this category. Breeding herds with very low PRRSV prevalence typically exhibit very mild or no clinical signs suggestive of PRRSV infection and have returned to their baseline levels of productivity. Replacement and breeding animals are expected to be negative for PRRSV by RT-PCR. All, or nearly all, breeding females are positive for PRRSV antibodies. Breeding replacements may be positive or negative for PRRSV antibodies. A vaccine is not used in any subpopulation of animals in the breeding herd, however modified-live virus vaccine or deliberate exposure to wild-type PRRSV (ie, live virus inoculation) may be used to acclimate breeding replacement animals as long as they are no longer actively shedding virus when they enter into the breeding herd. In herds where the goal is to control PRRSV, Category II may be the target herd status. When elimination of PRRSV is the goal, Category II is a transitional category for herds that are eliminating the virus by herd closure and rollover and, at some point, replacement animals that are naive to PRRSV would be introduced to move to Category III and eventually Category IV as the target herd status.

Category II-vx: Positive Stable With Vaccination

The criteria for promoting a herd into the Positive Stable With Vaccination (IIvx) category is similar to the criteria for promoting a herd in the Positive Stable (II) category. After 90 days of diagnostic testing to demonstrate a sustained lack of viremia of wild-type PRRSV in

weaning-age pigs, a herd may be promoted to Category II-vx. The defining characteristic of herds in Category II-vx is that piglets at weaning are consistently negative for PRRSV RNA by RT-PCR. In Category II-vx this requirement must be supported by a consistent lack of detection of wild-type PRRSV in serum from weaning-age piglets tested by RT-PCR and other validated molecular diagnostic methods to distinguish whether positive RT-PCR results were due to wild-type virus or vaccine-like virus (Table 1). As with Category II, testing of other sample types may be used to provide additional evidence but they cannot stand alone to promote or maintain a herd into Category II-vx.

The primary difference between Category II-vx and Category II is that replacement breeding animals, sows, and piglets may be immunized with a modified-live virus vaccine. If a modified-live virus vaccine is used on suckling piglets, diagnostic samples should be collected before administering the vaccine. Additionally, live virus inoculation, or any other administration of wild-type virus, may be used as an immunization strategy for replacement breeding animals to acclimate them before being entered into the breeding herd. If wild-type virus is used to inoculate sows in the breeding herd, the herd will achieve a status no higher than Positive Unstable, Low Prevalence (Category I-B). Detection of only modified-live vaccine virus is considered a negative result for the purpose of promoting a herd to a new category or maintaining a herd in a category. Any herd administering a modified-live virus vaccine may be given a grace period of two weeks post vaccine administration where any PRRSV-positive result by RT-PCR is assumed to be a detection of vaccine virus only. If, after the grace period, a sample from a vaccinated herd tests positive for PRRSV by RT-PCR, other molecular diagnostic methods, such as ORF-5 sequencing, whole-genome sequencing, or PCR clamping assays, may be used to distinguish whether positive RT-PCR results were due to wild-type virus or vaccine-like virus. If the ORF-5 sequence or other molecular diagnostic results indicate that the PRRSV isolate is vaccine-like, the result is considered negative for the purpose of promoting a herd to a new category or maintaining a herd in this category. If the ORF-5 sequence or other molecular diagnostic results indicates that the PRRSV isolate is a wild-type PRRSV, the result is

considered positive. Vaccinated herds typically exhibit transient minor or no clinical signs following vaccination events and have returned to their baseline levels of productivity. Replacement and breeding animals are expected to be negative for PRRSV by RT-PCR although occasionally may test positive to the vaccine virus that was used. All, or nearly all, breeding females are positive for PRRSV antibodies. Breeding replacement animals may be positive or negative for PRRSV antibodies. In herds where the goal is to control PRRSV with vaccination in the breeding herd, Category II-vx is the target herd status.

Category III: Provisional Negative

The Provisional Negative (III) category is unchanged from its initial description in the 2011 publication.¹ Category III is specific to herds that have eliminated PRRSV by herd closure and rollover, or similar methods. To demonstrate that PRRSV has been eliminated from the herd, PRRSV-naive breeding replacement animals, which serve as sentinels, must be introduced into the herd and remain seronegative by enzyme-linked immunosorbent assay (ELISA) at least 60 days following their introduction (Table 2). To serve as effective sentinels, the PRRSV-naive breeding replacement animals should have nose-to-nose contact opportunities and be housed in the same air space as the breeding females already in the herd. No animals in Category III herds are actively shedding virus, but they may have been exposed to the virus. Category III is a transitional category for herds that are eliminating the virus by herd closure and rollover, which will eventually advance to Category IV as the target herd status.

Category IV: Negative

This category also remains the same as described in the 2011 publication.¹ These herds have negative exposure and shedding status. The supporting diagnostic evidence for a herd to be promoted to Category IV is presented in Table 2. Category IV is the target status for herds that are eliminating the virus by herd closure and rollover or complete depopulation and repopulation with replacement animals that are naive to PRRSV. New herds stocked with animals that are PRRSV naive are also classified as Category IV.

Addition of supporting evidence required to stay in a category

In the original classification system, once a breeding herd achieved a status, additional evidence collected periodically was not required for a herd to remain in that category. Herds would generally only move to a lower category when a PRRSV outbreak occurred in the herd. However, it was the consensus of the working group that diagnostic testing should be done periodically to reconfirm that a herd remains in a category. Therefore, the supporting evidence to stay in a category was developed for all the categories. The supporting evidence to remain in Categories I-B, II, and II-vx is presented in Table 1 and the supporting evidence to remain in Categories III and IV is presented in Table 2. In the absence of supporting evidence to maintain a herd in any category, the default is the Positive Unstable, High Prevalence (I-A) category. No supporting evidence is required to maintain a herd in Category I-A.

Grow-finish classification

The working group also made a change to the classification of growing pigs published in 2011.¹ The new system is shown in Table 3 and features four categories: Positive; Seropositive, non-shedding; Vaccinated; and Negative. The system classifies a group of pigs at a point in time during the growing period, from weaning to market. Therefore, a single group of pigs may fall into more than one category during the growing period. The status of a group of pigs, as illustrated in this system, would be determined by testing 6 oral fluid samples collected from ropes geospatially distributed among all pens, barns, and rooms in which the group of pigs, as defined by the producer, are housed. In groups of growing pigs that are not vaccinated against PRRSV with modified-live virus vaccine, the status of the pigs may be determined by testing individual oral fluid samples for PRRSV antibodies by ELISA or other validated serological tests and for the virus RNA by RT-PCR. In groups vaccinated against PRRSV with a modified-live virus vaccine, the status of the pigs may be determined by testing individual oral fluid samples for the virus by RT-PCR. If a sample from a vaccinated group of pigs tests positive for PRRSV by RT-PCR, other validated molecular

Table 1: Summary of supporting diagnostic evidence required to promote and maintain a herd in PRRSV Categories I-B, II, and II-vx*

	Category	I-B		II and II-vx	
	Description	Positive Unstable, Low Prevalence		Positive Stable and Positive Stable With Vaccination	
	Testing purpose	To promote into	To maintain in	To promote into	To maintain in
	Animals and sample tested [†]	Serum from weaning-age pigs	Serum from weaning-age pigs	Serum from weaning-age pigs	Serum from weaning-age pigs
	Minimum number sampled	30 pigs	ive Unstable, PrevalenceImage: To maintain inTtoTo maintain inTigsSerum from weaning-age pigsw30 pigs30 pigs115 pigs/pool11Test pools by RT-PCR10Monthly or by batchd1One or more pools positive means herd test are negative, if <75%, revert to I-A1175% (3 of 4) of rolling monthly or batch herd tests are negative, if <75%, revert to I-A1dsProcessing fluidswersMajority of litters from one week of farrowing1is1 or more pools poils positive are negative, if <75%, revert to I-A2yTest pool(s) by 	60 pigs	30 pigs
	Pooling recommendation	5 pigs/pool	5 pigs/pool	10 pigs/pool	5 pigs/pool
	Test used	Test pools by RT-PCR		Test pools by RT-PCR	Test pools by RT-PCR
Option 1	Testing frequency [‡]	Monthly for 90 days or at least 4 batches		Monthly for 90 days or at least 4 batches	Monthly or by batch
	Herd test interpretation [¶]	One or more pools positive means herd test is positive	pools positive means herd test	One or more pools positive means herd test is positive	One or more pools positive means herd test is positive
	Requirement to promote or maintain status	75% (3 of 4) of monthly or batch herd tests are negative	PrevalencePositiPrevalencePositiPrevalencePositiPrevalencePositiPrevalencePositiPrevalencePositiPrevalencePositiPrevalencePositiPrevalence <t< td=""><td>100% (4 of 4) of monthly or batch herd tests are negative</td><td>Monthly or batch herd tests are negative If any positive, revert to I-B or lower</td></t<>	100% (4 of 4) of monthly or batch herd tests are negative	Monthly or batch herd tests are negative If any positive, revert to I-B or lower
	Animals and sample tested [†]	Processing fluids	Processing fluids	Concurrently; 1) Serum from weaning-age pigs 2) Processing fluids	Concurrently; 1) Serum from weaning-age pigs 2) Processing fluids
	Minimum number sampled	Majority of litters from one week of farrowing	from one week of	1) 30 pigs 2) Majority of litters from one week of farrowing	1) 30 pigs 2) Majority of litters from one week of farrowing
	Pooling recommendation	1 or more pools	1 or more pools	1) 5 pigs/pool 2) 1 or more pools	1) 5 pigs/pool 2) 1 or more pools
	Test used	Test pool(s) by RT-PCR		Test pool(s) by RT-PCR	Test pool(s) by RT-PCR
Option 2	Testing frequency [‡]	Weekly for 90 days or at least 4 batches		1) Monthly for 90 days or at least 4 batches 2) Weekly for 90 days or at least 4 batches	1) Quarterly 2) Monthly or by batch
	Herd test interpretation [¶]	One or more pools positive means herd test is positive	pools positive means herd test	One or more pools positive means herd test is positive	One or more pools positive means herd test is positive
	Requirement to promote or maintain status	75% (10 of 13) of weekly or batch herd tests are negative	rolling monthly or batch herd tests are negative, if <75%, revert to	 1) 100% (4 of 4) of monthly or batch herd tests are negative 2) 100% (13 of 13) of weekly or batch herd tests are negative 	1) Quarterly herd test is negative 2) Monthly or batch herd test is negative If any positive, revert to I-B or lower

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Table 1: Continued

	Category Description	I	I-B		ll and ll-vx	
		Positive Unstable Low prevalence		Positive Stable and Positive Stable with Vaccination		
	Testing purpose	To promote into	To maintain in	To promote into	To maintain in	
	Animals and sample tested [†]		Family oral fluids from litters of weaning-age pigs		Concurrently; 1) Serum from weaning-age pigs 2) Family oral flu- ids from litters of weaning-age pigs	
	Minimum number sampled		20 litters		1) 30 pigs 2) 20 litters	
	Pooling recommendation		5 litters/pool		1) 5 pigs/pool 2) 5 litters/pool	
	Test used		Test pools by RT-PCR		Test pools by RT-PCR	
Option 3	Testing frequency [‡]		Monthly or by batch		1) Quarterly 2) Monthly or by batch	
	Herd test interpretation¶		One or more pools positive means herd test is positive		One or more pools positive means herd test is positive	
	Requirement to promote or maintain status		75% (3 of 4) of rolling monthly or batch herd tests are negative, if <75%, revert to I-A		1) Quarterly herd test is negative 2) Monthly/batch herd test is negative If any positive, revert to I-B or lower	

* In the absence of supporting evidence to promote or maintain a herd in any category, the default is the Positive Unstable, High Prevalence (I-A) category. No supporting evidence is required to promote or maintain a herd in Category I-A.

[†] Processing fluids are collected from piglets seven days of age or younger. Weaning-age pigs are within seven days of weaning. Family oral fluids are collected from litters within seven days of weaning.

In herds where a multi-week batch-farrowing system is used, a single herd test is performed per batch. A herd test must be performed for at least 4 batches, even if more than 90 days is required to do 4 herd tests. For 3-week, 7-group batch farrowing systems, five herd tests should be conducted over 12 weeks (84 days) which is sufficiently close to 90 days.

A positive RT-PCR test result within 2 weeks of administration of modified-live virus vaccine in the herd is assumed to be detection of vaccine virus only and deemed a negative herd test for the purpose of the classification as Category I-B and II-vx. After the two-week grace period, other molecular diagnostic methods, such as ORF-5 viral sequencing, whole genome sequencing or RT-PCR clamping assays, may be used to distinguish whether positive RT-PCR results were due to wild-type virus or vaccine-like virus. If the ORF-5 sequence or other molecular diagnostics results indicate that the PRRSV isolate is vaccine-like, the result is considered negative for the purpose of promoting a herd into or maintaining a herd in Category I-B or II-vx.

PRRSV = porcine reproductive and respiratory syndrome virus; RT-PCR = reverse transcriptase-polymerase chain reaction; ORF-5 = open reading frame 5.

Table 2: Summary of supporting diagnostic evidence required to promote and maintain a herd in PRRSV Categories III and IV

	Category			IV	
	Description	Provisionally Negative		Negative	
	Testing purpose	To promote into	To maintain in	To promote into	To maintain in
Option 1	Animals and sample tested	Serum from PRRSV naive re- placement breed- ing animals that have been in herd for at least 60 days	Serum from PRRSV naive re- placement breed- ing animals that have been in herd for at least 60 days	Serum from adult breeding animals	Serum from adul breeding animals
	Minimum number sampled	60 animals	30 animals	60 animals	30 animals
	Pooling recommendation	None allowed	None allowed	None allowed	None allowed
	Test used	Test individual samples by ELISA	Test individual samples by ELISA	Test individual samples by ELISA	Test individual samples by ELIS/
	Testing frequency	Once	Semi-annually	Once	Semi-annually
	Herd test interpretation*	One or more posi- tive samples after ruling out false positives means herd test is positive	One or more posi- tive samples after ruling out false positives means herd test is positiveOne or more posi- tive samples after ruling out false positives means herd test is positiverdSemi-annual herdOne-time herd	tive samples after ruling out false positives means herd test is	One or more posi tive samples afte ruling out false positives means herd test is positive
	Requirement to promote or maintain status	One-time herd test is negative		Semi-annual hero test is negative, if positive, rever to Category I-B o lower	
	Animals and sample tested		Processing fluids from litters of PRRSV-naive re- placement breed- ing animals that have been in herd for at least 60 days		Processing fluids
	Minimum number sampled		for at least 60 days30 animals30 animalsNone allowedTest individual samples by ELISASemi-annuallyOne or more posi- tive samples after ruling out false positives means herd test is positiveSemi-annual herd test is negative, if positive, revert to Category I-B or lowerProcessing fluids from litters of PRRSV-naive re- placement breed- ing animals that have been in herd		Majority of litter from one week o farrowing
Option 2	Pooling recommendation		1 or more pools		1 or more pools
	Test used				Test pools by ELISA
	Testing frequency		Semi-annually		Semi-annually
	Herd test interpretation*		positive samples after ruling out false positives means semi- annual test is		One or more pos tive samples afte ruling out false positives means semi-annual tes is positive
	Requirement to promote or maintain status		test is negative, if positive, revert to Category I-B or		Semi-annual her test is negative, if positive, rever to Category I-B o lower

Table 2: Continued

	Category	III Provisionally Negative		IV Negative	
	Description				
	Testing purpose	To promote into	To maintain in	To promote into	To maintain in
	Animals and sample tested		Family oral fluids at weaning-age from litters of PRRSV-naive re- placement breed- ing animals that have been in herd for at least 60 days		Family oral fluids from litters of weaning-age pigs
	Minimum number sampled		20 litters		20 litters
	Pooling recommendation		None allowed		None allowed
Option 3	Test used		Test individual samples by ELISA		Test individual samples by ELISA
	Testing frequency		Semi-annually		Semi-annually
	Herd test interpretation*		One or more posi- tive samples after ruling out false positives means herd test is positive		One or more posi- tive samples after ruling out false positives means herd test is positive
	Requirement to promote or maintain status		Semi-annual herd test is negative, if positive, revert to Category I-B or lower		Semi-annual herd test is negative, if positive, revert to Category I-B or lower

* Serial testing using another antibody-based test with greater specificity may be used to rule out false positives.

[†] For herds that are eliminating the virus by herd closure and rollover, removal of all previously infected animals from the herd may be confirmed with production records. All breeding animals present in the herd on the first day the herd was classified as Category III are no longer on the list of animals inventoried.

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay.

Table 3: Classification of growing pigs for PRRSV status

Classification	ELISA status	Wild-type PRRSV RT-PCR status	MLV PRRSV RT-PCR status
Positive	+	+	+/-
Seropositive, non-shedding	+	-	-
Vaccinated	+	-	+
Negative	-	-	-

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; RT-PCR = reverse transcriptase-polymerase chain reaction; MLV = modified-live virus

diagnostic methods, such as ORF-5 viral sequencing, whole-genome sequencing, or PCR clamping assays, may be used to distinguish whether positive RT-PCR results were due to wild-type virus or vaccine-like virus. If the ORF-5 sequence or other molecular diagnostic results indicates that the PRRSV isolate is vaccinelike, the result is considered negative for the purpose of classifying the group of pigs.

Implications

- New system classifying PRRSV status of herds addresses developments since 2011.
- Value of system to classify PRRSV status of herds is evident in how it is used.
- Diagnostic testing is necessary to objectively classify herds for PRRSV status.

Acknowledgments

The authors acknowledge Maddie Herring for her assistance with the preparation of the manuscript. The AASV provided funding to support the working group and their work to propose modifications to the PRRSV classification system.

Conflict of interest

None reported.

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