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Uses of RT-PCR

Enric Mateu
Senior lecturer at the Department of Animal Health and Anatomy at the Faculty of Veterinary Medicine/Science (Autonomous University of Barcelona) Researcher at CReSA-IRTA

Cinta Prieto
Senior lecturer at the Department of Animal Health at the Faculty of Veterinay Medicines/ Science (Complutense University of Madrid)

Highlights

The direct detection of the nucleic acid of the virus by means of the RT-PCR has become one of the most frequent systems for the laboratorial diagnosis of PRRS outbreaks due to its high sensitivity and the limited utility of serology.

The diagnosis through RT-PCR has some limitations, such as: 1. Differences in sensitivity and specificity between laboratories; 2. The possible presence of inhibitors in the samples; 3. Problems in the detection of some strains as a consequence of the genomic variability of the virus; and 4. False positives problems due to cross contaminations between the samples.

The detection of the virus by means of the RT-PCR is useful for: the diagnosis of outbreaks in sows, the monitoring of artificial insemination centres, the establishing of the moment of circulation of the virus, together with serology, in the growing animals, and the monitoring of control programmes.

In order to understand the value of the detection of the virus in biological samples during the disease's diagnostic process, we need to know the dynamics of the infection. Right after the infection with PRRSV a period of viraemia is established, with a variable duration that depends, basically, on the age of the infected animals and on the virulence of the strain that causes the infection, facilitating the spreading of the virus through different organs and tissues. Once the viraemia has been solved, the virus can also persist for a long period of time in some of these organic locations, especially in the lymphoid tissue. Also, in the pregnant sows the virus can cross the placental barrier and replicate in the foetal side of the placenta causing the foetuses' death, even once the sow has gotten over the viraemia. In the same way, the virus can reach the reproductive system of the boar and be excreted in the semen. Besides, as the arrival of the virus to the semen is basically related to the migration of infected macrophages to the reproductive system, the virus can be excreted even in the absence of viraemia. All these enable the tackling of the disease's diagnosis by using methods that specifically establishes the presence of the virus in different biological samples.

Among the methods developed to detect the virus, we must underline the determination of the presence of its genetic material by means of molecular techniques that imply the reverse transcription (RT) of the viral ARN and the polymerase chain reaction (PCR). These techniques are characterised by obtaining of a

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high number of copies of the nucleic acid used as a template until an easily detectable amount is obtained, or even, depending on the technique used, easily quantifiable. Therefore, they allow detecting very small amounts of the nucleic acid that we are looking for in the initial sample, and thus obtaining a high sensitivity in the diagnosis. They are also very specific, because the amplification will only take place if the nucleic acid is contained in the sample that we wish to test, regardless of the number and amount of other nucleic acids present in the sample.

The high sensitivity and specificity of the RT-PCR techniques and the easiness of collection of blood or oral fluid samples have favoured the use of this diagnostic means. We must also consider as an additional advantage that serology has no diagnostic value in positive populations, and this forces the determination of the virus' presence in clinical samples from infected animals to reach a definitive diagnosis, and its establishment in the biological samples is the final evidence of its implication in the pathologic process observed.

Nevertheless, the advantages mentioned previously do not always appear in practice or give place to the disease's accurate diagnosis due to the following considerations; some, related to the technique itself and others to the dynamics of the infection, to be borne in mind.

Factors related to the technique:

1. Contrary to what happens with the ELISA techniques, that are mainly commercially available and are standardised, in the case of the RT-PCR, the sensitivity and specificity of the technique can vary considerably between laboratories. This is due to the fact that the carrying out of the assay implies the obtaining of the nucleic acid of the virus from the biological samples, the reverse transcription and the amplification of the nucleic acid by means of the PCR. For each and all of these steps, there is a wide variety of methodologies and manufacturers of different reagents that each diagnostic laboratory combines in different ways. Although recently, the use of different standardised commercial systems have become generalised, the sensitivity can vary considerably between them, and also, in some laboratories, home-made protocols designed by the laboratory itself, are used, and their sensitivity and specificity may be very variable.

2. Some biological samples contain inhibitors of the RT and PCR reactions, so if this fact is not borne in mind and specific systems that impede the action of these inhibitors in the samples are not applied, it is possible to obtain false negative results, limiting in this way the sensitivity of the technique. Some good examples are the semen or oral fluids samples that must undergo a different treatment than the serum samples, whose use is more generalised.

3. Finally, regarding the sensitivity of the technique, we must bear in mind that, contrary to what happens with the determination of antibodies by means of the ELISA technique, the very high variability of the PRRSV isolates makes the sensitivity of the technique deeply influenced by the homology between the sequence of nucleotides of the nucleic acid of the virus and that of the primers used in the assay. The design of the primers is, therefore, essential to guarantee a good sensitivity, and we must underline that the manufacturers of commercial kits for the detection of the virus in biological samples are constantly updating their techniques to optimise the sensitivity for the strains circulating in the field.

4. On the other hand, the high potential sensitivity of the technique makes the contamination between samples possible. We must bear in mind that the technique is based on the obtaining of a high number of copies of the nucleic acid of the virus from a very low number of copies. Therefore, if there is a high amount of virus in a sample, it is possible that a small amount of its nucleic acids reach other samples during the carrying out of the technique, or that it even contaminates the environment where the technique is carried out. This will lead to the obtaining of false positive results if we do not proceed with extreme caution.

On the other hand, and regarding the dynamics of the infection and the traits of the virus, the following aspects may lead to the obtaining of non-expected results:

1. The obtaining of positive results in infected animals requires an appropriate selection of samples, because only the samples in which the virus is present will render positive results. In this sense, we must underline that, for making a good design regarding the taking of samples, we must know intimately the dynamics of the infection. So, for instance, in the case of outbreaks of the disease in breeders, we must be aware that from the initiation of the infection until the appearance of the miscarriage, a lapse of time not less than a week goes by; generally between 10 to 15 days. This fact, together with the circumstance that the viraemia in adult animals can have a relatively short duration, especially in animals with a previous immunity, can lead to the appearance of a miscarriage in non-viraemic animals, which will lead to a negative result if we want to diagnose the cause of the miscarriage with a serum sample. On the contrary, the duration of the viraemia in young animals is relatively long, and this facilitates the detection of the virus in serum samples, even when the animals have been showing symptoms for several days.

2. The detection of the virus in boars deserves a special mention. In the boars it is very relevant to make an early and accurate diagnosis of the infection due to the epidemiologic repercussions of the excretion of the virus in the ejaculate. With time, different systems for the diagnosis of the infection in the boars have been developed. Among them, we must underline the persistence of the presence of the virus in blood samples, in semen samples or the determination of the seroconversion. Although it was thought, for many years, that the detection of the presence of the virus in semen samples was the most appropriate method for reaching the diagnosis of the infection, nowadays it is generally accepted that the best way to detect the infection in boars involves their monitoring and studying the viraemia. Nevertheless, if for whatever reason we decide to establish the presence of the virus in the semen, it is necessary to bear in mind that the amount of viruses present in the semen is normally lower than the amount that can be found in the serum, especially in the later stages of the infection, and that establishing the presence of the virus in semen samples is difficult due to the existence of polymerase inhibitors in the boars' ejaculate.

Based on all that has been mentioned, the RT-PCR would be the technique of choice for tackling the diagnosis of the disease in the following cases:

1. Sows. The outbreaks of the disease that include reproductive failure must be diagnosed by establishing the presence of the virus by means of RT-PCR on affected animals. Nevertheless, we must take into consideration that the viraemia is normally short and that it can end before the miscarriage happens. Therefore, a positive result confirms the diagnosis, but a negative one does not exclude the implication of the virus. Likewise, the testing of the miscarried foetuses frequently shows a negative result. This is due to two reasons: 1. the autolytic processes degrade the nucleic acid of the virus, hindering its amplification with the RT-PCR, and 2. not all the miscarried foetuses are infected. Therefore, we can conclude that, as in the previous case, a positive result confirms the diagnosis, but a negative one does not rule out the implication of the virus. Due to all this, one of the samples of choice for the diagnosis of the reproductive failure is the serum of weak-born piglets, because they are viraemic at birth and have lengthy viraemias.

2. Boars. In boars, the infection can be confirmed by means of establishing the viraemia or through the presence of the virus in semen samples. The main utility of the RT-PCR is the monitoring of the artificial insemination centres.

3. Growing animals. In nursery-stage pigs and fattening pigs, the RT-PCR can be used in addition to serology to determine the batches moment of the infection and to establish the corresponding control measures. Likewise, the determination of the presence of the virus in lung samples can be of use for confirming the role of the PRRSV in the porcine respiratory complex, even though the serum profiles can lend more valuable information in this case.

4. Control programmes. The RT-PCR can also be used to monitor the programmes' success on controlling the disease. It is useful to establish it if the sows' population is stable, confirming that the piglets are not viraemic at weaning, and also to guarantee that the programmes for the adaptation of the replacement sows established on the farms are carried out properly and are conducive to the infection of the sows when desired.

References

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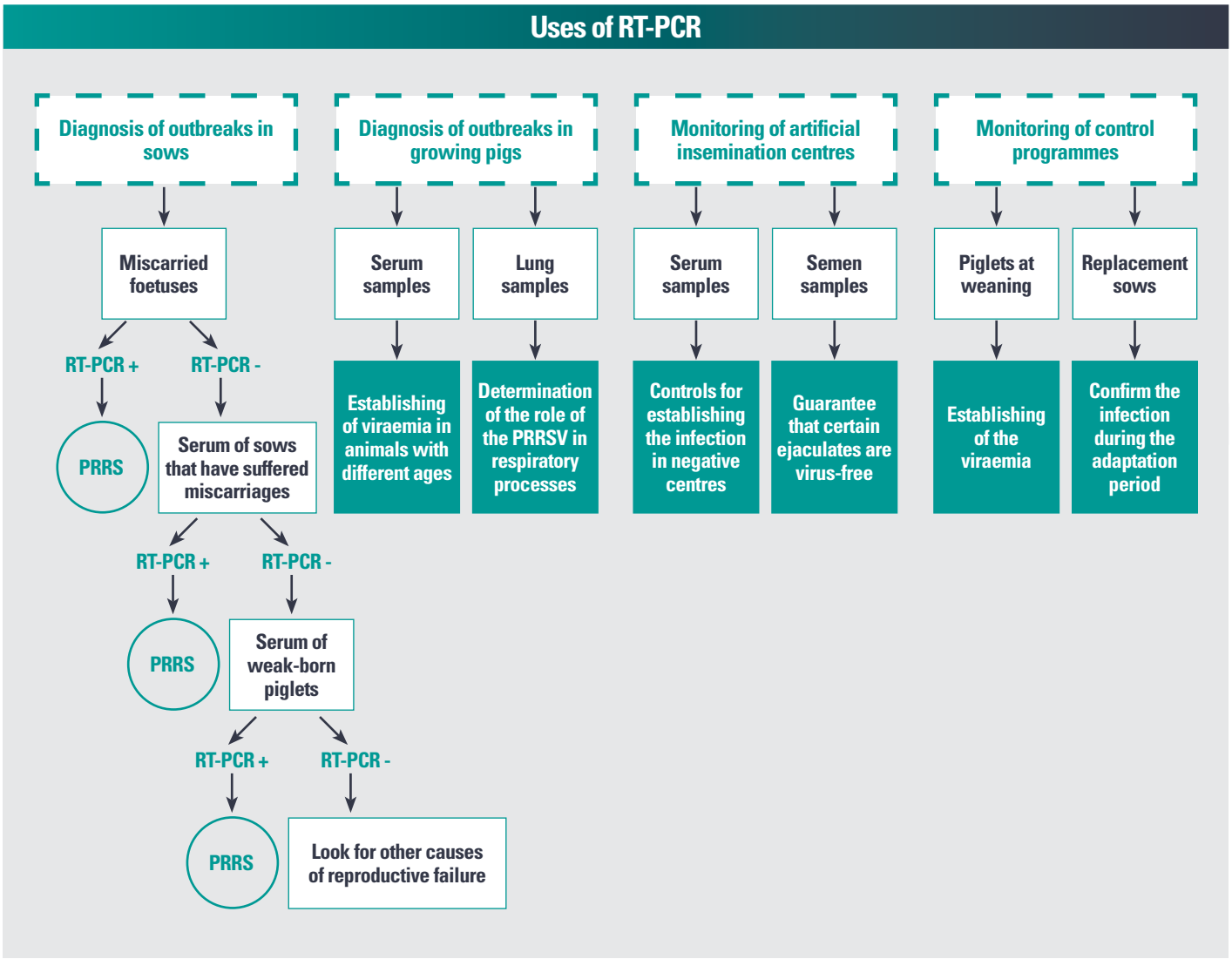
Figure 1.


Uses of RT-PCR
Diagnosis of outbreaks in sows
Miscarried foetuses
RT-PCR +
RT-PCR -
PRRS
Serum of sows that have suffered miscarriages
RT-PCR +
RT-PCR -
PRRS
Serum of weak-born piglets
RT-PCR +
RT-PCR -
PRRS
Look for other causes of reproductive failure
Diagnosis of outbreaks in growing pigs
Serum samples
Establishing of viraemia in animals with different ages
Lung samples
Determination of the role of the PRRSV in respiratory processes
Monitoring of artificial insemination centres
Serum samples
Controls for establishing the infection in negative centres
Semen samples
Guarantee that certain ejaculates are virus-free
Monitoring of control programmes
Piglets at weaning
Establishing of the viraemia
Replacement sows
Confirm the infection during the adaptation period