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EXPLORING POSSIBILITIES OF DIAGNOSING, CONTROL AND ERADICATION OF PORCINE RESPIRATORY AND REPRODUCTIVE SYNDROME (PRRS)**

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**Original scientific paper

Abstract: Porcine reproductive and respiratory syndrome is a pervasive disease that often seriously affects the health status of the animals and may lead to severe economic losses. Substantial efforts have been made to promptly diagnose this disease, and this paper presents an overview of clinical and laboratory investigation of PRRS in all animal categories within an industrial pig herd. Suspect disease was identified by clinical observation, and virus identification and detection of specific antibodies in suspect material were confirmed by laboratory examination. Isolation and identification of the virus from samples obtained from dead and sacrificed piglets was carried out on cell cultures (MARK-145) using fluorescence antibody technique, and molecular-biology techniques (RT-PCR and nestedPCR) were applied for detecting viral genome in native sperm samples. Presence of PRRS-specific antibodies was investigated in blood serum samples of boars by the use of ELISA. The virus was isolated from the suspect material of sacrificed piglet, and in two more sacrificed animals the virus was detected by the fluorescent antibody technique. The PRRS viral genome could not be detected in the native sperm of 10 boars even after triple sampling. Specific antibodies against PRRS were detected in 37 out of 42 examined boars.

Key words: PRRS, virus, diagnostics

Introduction

Porcine respiratory and reproductive syndrome (PRRS) is a viral, highly contagious disease that may affect all pig categories. The disease is manifested by various clinical symptoms; however, it may sometimes persist

without any clinical signs. PRRS is a relatively new disease. It was first observed as recently as 1987 in the state of Iowa, USA, (1;10;12;13). In Europe, the first case was reported in Germany in 1990 (10;12;13). Detection and identification of the causative agent have been the issues of paramount importance over almost 4 years in leading European and American laboratories. Finally, in 1991 the Central Veterinary Institute, Lelystad, the Netherlands, identified the virus as the causative agent of the disease (10). Initially, prior to detection of the causative agent, the disease was differently named. Presence of characteristic symptoms such as ear cyanosis or severe outcomes such as massive abortions and high morbidity and mortality rates brought names such as "blue ear disease" or "mystery swine disease". After detection and identification of the causative agent, the name *porcine respiratory and reproductive syndrome* (PRRS) was proposed by the American and European researchers. The disease brings severe health problems and substantial economic losses. An area in Iowa suffered loss of some 85.000 pigs during only one season, and in winter 1990/91 nearly one million pigs died in Western Europe. Losses resulting from this disease are estimated to already overwhelm the total losses caused by Aujeszky`s disease so far (14). Some financial analyses report on direct losses of 70 to 350 $ per one sow. If taking in account the potential loss of profit, the calculated loss per one sow increases up to 450 $ (10).

The causative agent of the PRRS is a virus characterized by numerous biological traits. The viral genome has a single-stranded RNA of only 15 kb pairs, i.e. only eight operons, which is responsible for its pronounced genetic variability. An unstable genome and incomplete replications, i.e. frequent mutations, resulted in extensive variability of the isolates. However, the isolates are classified into the two major PRRS genotypes, the North American and the European one. The North American genotype encompasses isolates from Asia (Japan, China, Taiwan), while the European genotype includes isolates from Germany, Spain, Netherlands and Denmark. European genotype closely resembles to isolates from Russia. There are numerous reports on isolation of PRRSV in America that closely resembles the European genotype and vice versa. The molecular divergence between the European and North American PRRSV range between 21% and 48%; however the researchers have postulated that both genotypes are derived from a common ancestor. The diversity of virus isolates is mirrored not only in their amino-acid composition of their glycoproteins, but primarily in their infective and pathogenic potential. Their infectivity and pathogenicity significantly varies with respect to the pig category. However, the PRRSV

isolates exhibit some common features, i.e. their genome organization and replication strategy are identical. Furthermore, pigs are the only known PRRSV susceptible species. The infected animal may be able to shed the virus in the feces and urine for 28 days, in saliva for 42 days and in semen for 92 days after the onset of original infection. However, the virus persists and remains active in the blood, tonsils and muscles of infected animals for 210, 157 and 7-14 days, respectively. The infective potential of the virus is highly unstable in the environment and is determined by the temperature and pH. The virus is mostly preserved at low temperatures, while high temperatures have cidal effect. At -20^0 C the infective potential of the virus is preserved for several years, while temperature $+ 56^0$ C inactivates the virus within some 10 minutes. The most suitable pH value for virus survival ranges between pH 6,5 and 7,5 and each change may result in quick inactivation of the virus. The virus is mostly spread by infected animals, via their excreta, as well as by the farm equipment, such as injection needles and syringes, fixation equipment, etc. Operatives at the farm could present and important source of infection if not obeying appropriate veterinary-sanitary and zoohygienic measures. In infected animals the virus replication predominantly takes place in the lung macrophages, monocytes and capillary endothelium, hence pathogenesis of PRRS is to be regarded from the aspect of the damage and dysfunction of these cells.

Clinical symptoms occurring in affected animals are different, depending on the virulence of the virus, age of the animal, presence of secondary pathogenic organisms, as well as on zoohygienic and zootechnical conditions in the herd. The infection can, however, remain asymptomatic, particularly in persistently infected animals.

Diagnostics of PRRS is highly specific issue that requires a systematic approach of the clinician, highly experienced and trained virologist, as well as well equipped laboratory facilities. A thorough clinical examination can identify the suspect disease with the high rate of probability; however, laboratory examination detecting the virus and specific antibodies is essential for the final diagnosis.

The aim of this study is to describe the obtained results of clinical and laboratory examination in the case of suspected infection with PRRS virus. The selection of the examined materials and research methods was not only aimed at detection of the infection, but also at its control. Our goal was to explore possible ways and procedures for eradication of this infection in industrial pig herds.

Material and methods

The investigated herd:

In an industrial pig herd presence of PRRS virus was suspected. Thorough clinical examination of all diseased animals was performed, revealing presence of the disease in all animal categories. Massive deaths were observed only in piglets in the pig raising units. Epizootiological survey identified the introduction of breeding boars into the herd as the possible source of infection. In that respect, serological examination encompassed all boars in the herd, and semen samples were collected from 10 randomly selected seropositive boars for the purpose of confirming presence of the virus and its transmission via the semen.

Material for laboratory examination:

Altered lung portions and lung lavages, mediastinal lymph nodes, tonsils and spleen from 16 dead and 8 diseased and euthanized piglets were used for virus isolation. Blood samples for serological testing were obtained from all 47 boars in the herd. Native sperm samples obtained from 10 randomly selected boars were used for virus isolation. Triple sampling was performed by obtaining three subsequent ejaculates according to the artificial insemination procedure. A total of 30 native semen samples were collected.

Isolation and identification of the virus:

An attempt to isolate the virus from suspect material obtained from piglets was made using cell culture MARK-145. The material was previously processed according to virology test procedures. The material was cultured onto the MARK-145 cell culture through three 7-day-passages. The cells from the third passage were examined applying direct immunofluorescence assay, the conjugate of anti- PRRSV polyclonal antibodies.

Serological diagnostics:

Titer values of PRRSV specific antibodies in blood sera of boars were determined using ELISA technique and the ELISA kit supplied by IDEEX Laboratories (USA).

Virus detection using biomolecular techniques

Presence of the nucleic acid of PRRSV genome was established using the nestedPCR test. Extraction of RNA was performed in 1.5 ml microtubes using Trizol® reagent (Gibco BRL) according to producer's instructions. The obtained RNA was subjected to one-step RT-PCR reaction taking place in the same tube, using the commercial kit Access RT-PCR system, Promega, U.K. In this reaction primers specific for preserved ORF 7 part of the viral

genome were used. The obtained PCR products from the RT-PCR test were used as samples in nestedPCR test, applying "HotStar Taq Master Mix Kit" (Qiagen, Germany). Detection of specific products (304 bp in RT-PCR and 209 bp in nestedPCR test) was performed on 1,5% agarose gel.

Results and discussion

Results of clinical investigation of PRRS obtained after comprehensive analysis of anamnestic data and clinical examination are presented in tables, with respect to particular pig categories.

Table 1. Prevalence of clinical symptoms (in percents) in sows in farrowing house

No.	Clinical symptoms and their prevalence (%)	
1	Anorexia	80
2	Agalactia	50
3	Hyperthermia	25
4	Cyanosis (ears, udder, tits, vulva)	0
5	Sudden deaths	0
6	Pareses and paralysis	0,3
7	Abortions from 90 – 110 days of gestation	0,5
8	Less live-born piglets	3
9	Dead suckling pigs	23
10	Anestria	0

According to literature data (10, 13) anorexia and agalactia are frequent symptoms of PRRS. Anorexia can occur in even 50%, and agalactia in 10% of sows. We must, however, emphasize that daily temperatures were extremely high and ventilation systems in farrowing houses did not function properly throughout the period of clinical survey of this herd, hence these factors are to be taken into account by identifying the causes of anorexia and agalactia. The sows brought forth the expected number of vital piglets, and increased number of dead piglets was considerably influenced by the health status of sows. Abortions at gestation period from 90-110 days that are considered crucial symptom of the reproductive form of the disease were observed just sporadically.

Analysis of clinical symptoms in the raising unit revealed high prevalence of symptoms of the respiratory syndrome in most piglets. Environmental conditions as well as impact of numerous pathogenic organisms are considered predominant etiological factors likely to induce

massive manifestation of respiratory syndrome symptoms.

Table 2. Prevalence of clinical symptoms (in percents) in piglets in raising units

No.	Clinical symptoms and their prevalence (%)	
1	Diseased piglets	60
2	Anorexia	60
3	Hyperthermia ($39,5^0$ C and more)	13
4	Rapid breathing	60
5	Painful and convulsive cough	40
6	Eyelid swelling	60
7	Conjunctivitis	50
8	Rhinitis	60
9	Course of the disease	21 days
10	Mortality	15

The eye-lid swelling associated with bristled hairs in the orbital region can be accentuated as a highly specific symptom observed in the greatest part of investigated piglets. According to literature data (6) within the range of PRRS symptoms, these symptoms are observed in 5% of piglets.

Table 3. Prevalence of clinical symptoms (in percents) in fattening pigs

No.	Clinical symptoms and their prevalence (%)	
1	Diseased animals	50
2	Anorexia	50
3	Lethargy	80
4	Cough	50
5	Mortality	2

Predominant symptoms recorded in a fattening unit were extreme decrease in feed intake (the feed consumption was reduced for almost 2/3), lethargy in most animals and frequent coughing. Literature data report on the prevalence of these symptoms in PRRS diseased fattening animals ranging from 10 to 25%.

Fatigue, lethargy and feed refusal was observed in almost all boars, and loss of libido in some 20% of boars. The data from the literature indicate that these symptoms may occur in PRRS-diseased boars; however, not in more than 25% of cases.

Analysis of the observed clinical symptoms in all age categories revealed substantial prevalence of symptoms of the respiratory syndrome that may suggest presence of PRRS. Prevalence of symptoms of reproductive form of

the disease was minimal, i.e. negligible. However, setting of final diagnosis, i.e. confirming of PRSS on the basis of manifest clinical symptoms was extremely difficult. Frequent occurrence of enzootic bronchopneumonia in piglets in raising units within this herd was induced by numerous etiological agents. In that respect and in accordance with recommendations of many pathologists PRRS is to be analyzed and evaluated within the complex of enzootic bronchopneumonia (1).

Table 4. Prevalence of clinical symptoms (in percents) in boars

No.	Clinical symptoms and their prevalence (%)	
1	Diseased animals	80
2	Anorexia	80
3	Lethargy	80
4	Cough	5
5	Loss of libido	20
6	Testicle swelling	0
7	Lowered sperm output	5
8	Mortality	0

Multiple culturing of the suspect material on MARK-145 cell culture demonstrated the cytopathogenic effect corresponding to PRRS virus. The observed cytopathogenic effect was manifested by culturing lung sample of sacrificed piglet. Positive finding to the PRRS virus was confirmed in a direct immunofluorescence assay using MARK-145 cell culture after culturing lung and mediastinal lymph node samples obtained from another 2 sacrificed piglets. Isolation of PRRS virus using cell cultures may often fail. Many virus strains have reduced potential of replication *in vitro* or, even if replication occurs the cytopathogenic effect in the cells is not manifested.

Results of serological diagnostics, i.e. the determined PRRSV-specific antibody titer values in boars are displayed in Table 5.

Table 5. Distribution of PRRSV antibody titer values determined by the use of ELISA

n = 42	Distribution of determined antibody titer values				
	< 900	900 – 3000	3001-6000	6001-9000	9000 i >
No. of animals	5	13	15	6	3
%	11,90	30,95	35,71	14,29	7,14

Antibody titer values below 900 are considered negative result, and they were determined in 5 investigated boars. Positive serological finding was obtained in 37 boars, yet with different antibody titer values. The determined antibody titer values ranged between 918 and 10375. Such immune reactivity implies that PRRSV infection did not occur simultaneously in all boars or that reinfection course has a cyclic pattern.

Presence of the virus genome could not be confirmed in semen samples obtained from 3 subsequent ejaculates of 10 randomly selected boars neither by the use of bio-molecular assays, nor by RT-PCR and nestedPCR. Such results imply that virus was not excreted in ejaculates used for sampling. However, it can not be explicitly stated that virus is not excreted in the semen of these boars. It is highly probable that virus is shed in the semen at particular stage of infection and spread within the herd.

Conclusion

Clinical and laboratory diagnostics of PRRS is a highly complex process requiring much experience of all subjects involved, as well as well equipped laboratories. During our clinical investigation the following symptoms were most frequently observed in piglets: anorexia, rapid and destructed breathing, conjunctivitis, rhinitis and Eyelid swelling. These symptoms were registered in more than 50% of piglets, while painful and convulsive cough was observed in 40% of investigated animals. In adult animal categories (sows, boars and fattening pigs) anorexia, lethargy and cough were predominant symptoms determined in more than 50% of animal population.

Laboratory diagnostics must encompass detection of the presence of the virus (detection of viral genome, its isolation and identification) and detection of specific antibodies. In the suspect material obtained from 16 dead and 8 sacrificed piglets the virus was isolated in MRFK-145 cell culture from the lungs of one sacrificed piglet. Using the fluorescence antibody technique by passaging the examined material in cell culture the virus was detected in several specimens from another 2 sacrificed piglets. Presence of PRRSV specific antibodies was confirmed in 37 (88,1%) boars in the herd, using ELISA technique. The virus could not be detected in triple semen samples of 10 boars by applying bio-molecular diagnostic techniques (RT-PCR and nestedPCR)

ISPITIVANJE MOGUĆNOSTI DIJAGNOSTIKOVANJA,KONTROLE I ISKORENJIVANJA RESPIRATORNOG I REPRODUKTIVNOG SINDROMA SVINJA (PRRS)

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Rezime

U zapatu svinja industrijskog načina odgoja sprovedena su klinička i laboratorijska ispitivanja respiratornog i reproduktivnog sindroma svinja (PRRS). Kliničkim ispitivanjima bile su obuhvaćene sve kategorije svinja. Kod prasadi najčešće utvrđeni simptomi su bili: anoreksija, ubrzano i otežano disanje, konjunktivitis, rinitis i otok očnih kapaka. Ovi simptomi utvrđeni su kod više od 50% prasadi, dok je bolan i grčevit kašalj utvrđen kod 40% posmatranih prasadi. Kod krmače, nerastova i tovljenika dominirali su sledeći simptoma bolesti: anoreksija, letargija i kašalj. Ovi simptomi su utvrđeni kod više od 50% ovih životinja.
Laboratorijska dijagnostika u postupku dokazivanja oboljenja mora da bude zasnovana na dokazivanju prisustva (dokazivanju genoma virusa, njegova izolaciji i identifikacija) i dokazivanju specifičnih antitela. U suspektnim materijalima uzetih od 16 uginulih i 8 žrtvovanih prasadi virus je izolovan na kulturi ćelija, MARK-145, iz pluća jednog žrtvovanog praseta, a tehnikom fluorescentnih antitela, pasažom ispitujućih materijala preko kulture ćelija, virus je dokazan u više materijala kod 2 žrtvovana praseta. Prisustvo specifičnih antitela protiv virusa PRRS ELISA tehnikom dokazano je kod 37 ili 88,1% nerastova u zapatu. Molekularnobiološkim dijagnostičkim tehnikama (RT-PCR i nestedPCR) nije dokazano prisustvo virusa u trokratno uzetim uzorcima sperme 10 nerastova.

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