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DEVELOPMENT OF A VARIANT OF DIRECT IMMUNOFLUORESCENCE TECHNIQUE IN THE DIAGNOSIS OF PRRS

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease spread in intensive growth with endemic evolution and with economic significance. In Romania the disease was for the first time diagnosed in 1998 and nowadays the disease is prevalent in many farms of pigs. For detection of viral antigens were sampled lymph nodes with pathological lesions macroscopic characteristic of the PRRS, from cadavers of piglets from farms where the disease evolves, and from pigs with clinical evolution of the disease was taken oro-nasal fluid. Viral nucleocapsid antigen was detected using kit - Anti PRRSV monoclonal antibody labelled with fluorescein isothiocyanate -BIO 268. The cytoplasm of cells, infected with PRRS virus, had a brilliant greenish yellow appearance due to the presence of the viral nucleocapsid antigens coupled with monoclonal antibodies labeled with fluorescein. Epithelial cells were rare, smaller, and fluorescent appearance of the cytoplasm was very evident. In case of the oro-nasal fluid smears, in the microscopic field were highlighted cells agglomerations with highly fluorescent cytoplasm. 19/30 (63.33%) of the examined samples (lymph nodes and oro-nasal fluid) were positive, respectively, 12/30 (40%) of the lymph nodes and 7/30 (23.33%) of oro-nasal fluid. Samples of lymph nodes and oro-nasal fluid were examined also through RT-PCR in order to create a correlation between the results provided by direct immunofluorescence (DIF) and RT-PCR, regarded as the reference method. The results confirm that the DIF can be adapted but more research is required to establish the sensitivity and specificity of this method.

Key words*: DIF, lymph nodes, oro-nasal fluid, PRRS.*

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease spread in intensive growth with endemic evolution and economic significance.

In Romania, the disease was for the first time diagnosed in 1998, and nowadays the disease is prevalent in many pigs' farms worldwide (Stanuica, 1999, 2005; Zimmerman et al., 2012).

Since 1998, several PRRS studies have been conducted in Romanian pig farms, some of them focused in serological evaluation of virus circulation by using ELISA (Stanuica, 1999; Baraitareanu et al., 2009; Campeanu et al., 2010), and other in detection of PRRS virus RNA by different RT-PCR techniques (Zaulet et al., 2011; Fluerașu et al., 2016).

The PRRS syndrome has rapidly expanded in intensive rearing pigs farm, where is producing significant economic losses.

This syndrome is produced by a ribovirus, placed in the *Arteriviridae* family, genus *Arterivirus*, with two genotypes respectively, Type 1 (European) and Type 2 (American) with a considerable variability of gene sequences, with similarity of only 50-60% (Stanuica, 2005; Zimmerman et al., 2012).

Confirmation of PRRS syndrome can be made (based) through several laboratory exams that aim detection of the virus, characterization of the isolated strains and antibody detection. For the rapid diagnosis techniques are preferred the immunohistochemical and immunofluorescence exams, who detects viral nucleocapsid antigen existing in the cytoplasm of infected cells in the lymph nodes, lungs and other organs (Mengelling et al., 1995; Botner, 1997; Zimmerman et al., 2012).

The immunofluorescence reaction (DIF) use monoclonal antibodies marked with fluorochromes for the detection of viral antigen from cryosections made it in lungs and lymph nodes (Botner, 1997; Jing et al., 2009; Zimmerman et al., 2012).
The aim of study was evaluation of a DIF as a rapid diagnostic method by using fingerprint smears of lymph nodes and oro-nasal fluid from pigs with clinical or pathological suspicion of PRRS.

MATERIALS AND METHODS

For detection of PRRS virus (PRRSV), 20 lymph nodes were sampled from piglet cadavers with characteristic lesions of PRRS. From pigs with respiratory syndrome was taken 10 oro-nasal fluid samples. All cadavers and oro-nasal fluid samples were supplied by a pig farm with a recent history of PRRSV infection.
Because the use of cryosections requires an endowment with cryotome, in the framework of research, DIF technique has been adapted in order to use the fingerprint smears made from lymph nodes and oro-nasal fluid.
Viral nucleocapsid antigen was detected using the commercial diagnostic test BIO 268 (Bio-X Diagnostics, 2016), a reagent for detection of Porcine Respiratory Reproductive Syndrome Virus: anti PRRSV monoclonal antibody labelled with fluorescein isothiocyanate.
DIF technique consists of the following steps: degreasing glass coverslips with ethyl alcohol exhibiting of samples of lymph fingerprint, desiccation of the wells and fixing in acetone for 15 minutes, desiccation of the wells for two hours, washing the slides with PBS-Blue Evans, and the desiccation of the wells. Oro-nasal fluid samples were centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant was siphoned off and the sediment transferred to a microscope slide.
Fluorescein-conjugate BIO 268 (0.1 µl) was added on dried and fixed slides, after which the slides were incubated for 1 hour at 21℃, and finally examined under the microscope with fluorescent light OLYMPUS.
PRRS virus RNA detection has been done by using RT-PCR technique developed at SN Institute Pasteur SA Bucharest (Romania), and previously described (Fluerașu et al., 2016). For this purpose were used four extraction kits (Qiagen and Roche, Germany) and two primers specific to ORF 7 area (Fluerașu et al., 2016).

RESULTS AND DISCUSSIONS

Inguinal lymph nodes were taken from fresh corpses of piglets, from primary outbreaks. Lymph nodes were swollen, oedematous, with firm consistence, and red on the section. Inguinal lymph nodes were preferred because they present macroscopic lesions well cast.
The results of DIF and RT-PCR exams are shown in Table 1.

Table 1. DIF and RT-PCR results

No.	Type	DIF		RT-PCR	
		+	–	+	–
1	lymph node	+		+	
2	lymph node	+		+	
3	lymph node		-	+	
4	lymph node		-	+	
5	lymph node	+		+	
6	lymph node		-	+	
7	lymph node	+		+	
8	lymph node		-	+	
9	lymph node		-		-
10	lymph node	+		+	
11	lymph node	+		+	
12	lymph node		-	+	
13	lymph node	+		+	
14	lymph node	+		+	
15	lymph node	+		+	
16	lymph node		-	+	
17	lymph node	+		+	
18	lymph node		-		-
19	lymph node	+		+	
20	lymph node	+		+	
21	oro-nasal fluid		-		-
22	oro-nasal fluid	+		+	
23	oro-nasal fluid	+		+	
24	oro-nasal fluid	+		+	
25	oro-nasal fluid	+		+	
26	oro-nasal fluid	+		+	
27	oro-nasal fluid	+		+	
28	oro-nasal fluid		-		-
29	oro-nasal fluid	+		+	
30	oro-nasal fluid		-		-

On the smears made from lymph nodes, the image from microscopic field was different being viewed isolated cells, cells grouped, or large clusters of cells. In case of positive samples, microscopic field predominated in medium and small lymphocytes, and large lymphocytes, plasma cells, and epithelial cells were rare. On medium and small lymphocytes, cell outline was obviously the nuclei were well individualized, the ratio between nucleus and cytoplasm was about equal. Large lymphocytes have a similar aspect, the plasma had an

elongated oval shape and cytoplasm was dominant.
The cytoplasm of cells, infected with PRRSV had a brilliant greenish yellow appearance due to the presence of the viral nucleocapsid antigens coupled with monoclonal antibodies labelled with fluorescein. Epithelial cells were rare, smaller, and fluorescent appearance of the cytoplasm was very evident.
In case of the oro-nasal fluid smears, in the microscopic field were highlighted cells agglomerations with highly fluorescent cytoplasm.
Smears made from lymph nodes and the oro-nasal fluid, when was identified in microscopic field the described aspects, were considered positive. Thus, 19/30 (63.33%) of the examined samples (lymph nodes and oro-nasal fluid) were positive, respectively, 12/30 (40%) of the lymph nodes and 7/30 (23.33%) of oro-nasal fluid.
Samples of lymph nodes and oro-nasal fluid were examined also through RT-PCR in order to create a correlation between the results provided by direct immunofluorescence and results from RT-PCR, regarded as the reference method.
The results obtained by RT-PCR were communicated through a previously published scientific paper (Fluerașu et al., 2016).
Samples of lymph nodes, and oro-nasal fluid confirmed as positive by direct immunofluorescence technique were confirmed as positive also by RT-PCR technique. Six DIF-negative samples of lymph nodes and oro-nasal fluid were confirmed as positive by RT-PCR technique, and five samples were negative in both techniques.
Until now, DIF was used only for nucleocapsid antigen detection of PRRSV by using cryosections from lymph nodes, lungs and other lymphoid organs. In this research was aimed adaptation of DIF method in order to be taken like a quick method of PRRS diagnosis using fingerprint smears made from the lymph fluid or oro-nasal sediment (Botner, 1997; Jing et al., 2009).
The results confirm that the DIF technique can be adapted but more research is required to establish with certainty the sensitivity and specificity of this method.

CONCLUSIONS

The operating-method, allowed us to obtain smears from lymph nodes and oro-nasal fluid, which with the aid of monoclonal antibodies fluorescein-labeled could be detected different types of cells infected with PRRS virus.
The IFD reaction made it by the methodology described, detected the presence of viral antigens in 63.33% of samples.
For using IFD as a rapid diagnostic method, is necessary to continue the researches on a larger number of samples and compared with more diagnostic methods.

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