THE INFLUENCE OF SOME MICROBIAL IMMUNOSUPRESIVE AGENTS ON THE EFFECTIVNESS OF IMMUNOPROPHYLAXIS PROGRAMS APPLIED IN BREEDING PIGS

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Abstract

This paper presents data obtained from investigations made in pig farms with intensive growing system and from households, the presence and distribution of immunosuppressive pathogens circulating in swine populations in Romania (PRRS, M. hyopneumoniae, Circovirus, etc.) and their immunosuppressive influence on the effectiveness of immunoprophylaxis programs, applied in pig farming.
The benefit of obtaining such data is to obtain and apply some effective immunoprophylaxis methods in order to limit economic losses and increase the food quality and safety.
The influence of the mentioned germs on the anti-erysipelas immune response has taken into account because this vaccination is a currently made action applied in the current technology of growing pigs.
Preliminary results showed that there is some influence of studied immunosuppressive microbial agents including the association germs on the effectiveness of immunoprophylaxis programs applied in breeding pigs.

Key words: *Circovirus, Mycoplasma, PRRS.*

INTRODUCTION

This paper is a study performed in intensive pig farms and households in our country regarding the existence of some pathogens (PRRS, Circovirus, Mycoplasma) and their immunosuppressive influence on the effectiveness of immunoprophylaxis programs, applied in pig farming. The choice of this topic was justified by the fact that there are worldwide encountered pathogens known to have immunosuppressive activity in pig populations.
Some pathogens present in the world, are known to negatively modulate the immune system, significantly interfering with the effectiveness of any vaccination protocol. Such pathogens are Porcine Circovirus type 2 (PCV-2) - major causative agent of Post Weaning Multisystemic Wasting Syndrome

(PMWS) (Gordon et al., 2007; Raymond et al., 1995), PRRS virus - causative agents of Porcine Reproductive and Respiratory Syndrome (Perianu, 2011; Herman et al. 2010), Mycoplasma hyopneumoniae - the causative agent of porcine enzootic pneumonia (PEP) (Perianu, 2011; Silin 2001).
Clinical signs of infection caused by mentioned germs are varied, being dependent on the immune status, conditions of farm hygiene and the presence of other pathogens.
The aim of our research was to obtain data on the influence of immunosuppressive pathogens (PRRS, porcine Circovirus, Mycoplasma) on the effectiveness of immunoprophylaxis programs applied in pig farming. For this reason it was studied the immunological response after anti-erysipelas vaccination in pigs, both *in intensive pig farms and households. The* influence of the mentioned germs on the anti-erysipelas immune response has taken into account because this vaccination is included in vaccination programs performed in pig farms.
Knowing these mechanisms enables us to understand the limits that appear in certain vaccinations in pigs and also enables us to think of the development and implementation of effective immunoprophylaxis methods in order to limit economic losses and to increase the food quality and safety.

MATERIALS AND METHOD

Biological material studied was the clinically healthy pigs of different ages. Animals came from three different locations (A, B, C) of pig growing: 2 intensive farms (Farm A: 172 animals Farm B: 90 animals) and households (50 animals).
These three farms performe different pig growing systems.
In the farm A is used an industrial (intensive) breeding of the young piglets imported from abroad (Holland) and also acquired from the local farmers. The piglets are acquisitioned at 65-75 days old and the growing is carried out up to 150-160 days old, when the pigs are delivered. During this period piglets are vaccinated against erysipelas and PRRS diseases. The serological surveillance is carried out for classical swine fever.
In the farm B is performed a growing system based on the close circuit (breeding, growing and fattening), the breeding material (gilts and young boars) being obtained from the own breeding material. In the past when piglets were acquired from abroad, the farm passed through a disease

occurrence, being declared as PRRS contaminated farm. The sanitary-veterinary surveillance is made for classical swine fever, Aujeszky disease and brucelosis. The self-control is performed by serological tests for PRRS and Circovirus, and also by anatomo-pathological examinations. In this farm is performed the vaccination against erysipelas in pigs of 90-120 days old, sows at 3 days after farrow and at 3 days before weaning.

In the small farm C (of the households), the growth system is extensive, the piglets are acquired from diferent places, animal markets or from the own breeding. In these farms are not performed any type of vaccinations.

The mortality and morbidity parameters recorded in the 3 different locations were in the technological limits; during the period of investigation were not recorded infectious diseases.

By ELISA test, were carried out serological examinations, both for detection of the specific antibodies against *PRRS, M. hyopneumoniae, Circovirus*, and for presence of erysipelas antibodies, before and after vaccination against this disease.

Serological examinations were performed on blood samples (from the jugular confluence) before and after anti-erysipelas vaccination. Pathological and bacteriological examinations from corpses were also performed.

540 blood serum samples obtained from 312 pigs were serologicaly analyzed: 252 young pigs (1.5 - 4 months), 30 fat pigs (6 months - 1 year) and 30 sows. Detection of specific IgG antibodies in blood serum (seroprevalence) was performed using ELISA kits for: PRRS (HerdCheck-PRRS X3 IDEXX Laboratories, USA), Circovirus type 2 (Porcine Circovirus (PCV2) ELISA Test Kit Green Spring, Shenzhen Lvshiyuan Biotechnology Co.., Ltd), Mycoplasma hyopneumoniae (M hyo * CHECK Herd, IDEXX Laboratories, USA) and Erysipelothrix rhusiopathiae (Erysipelothrix rhusiopathiae SE / MR, Cypress Diagnostics). Results were read at wavelengths recommended by manufacturers.

Interpretation of positive results was based on the S / P ratio greater than or equal to 0.4 (PRRS and M. hyo), the value of optical density (OD630) greater than or equal to 0.4 (PCV2) and relative index value x 100 (IRPC) greater than 40 (Erysipelothrix rhusiopathiae).

By anatomopathological examination performed on young pig carcasses were identified different lesions: ecchymosis and petechia in lungs, broncho-pneumonia, enlarged spleen, enlarged and hemorrhagic lymphnodes, catharal and catarrhal-congestive enteritis (Baba, 1996).

In order to isolate and identify the germ of the lesions, bacteriological examinations were made using specific culture media.Isolated strains were characterized biochemically using the API Biomerieux multitest systems. Antibiotic susceptibility testing of the pathogenic bacterial species was performed by antibiogram (Kirby-Bauer - diffusion method).

RESULTS AND DISCUSSIONS

Results of serological examinations:

In the **FARM A** were studied 6 groups of animals; group 1, group 1a, group 2, group 4, group 5a, group 5b, group 6, group 6a.
In the **group 1**, with 50 unvaccinated animals of 65 days old, the ELISA test results were: 49 positive to PRRS virus, 50 negative and 21 suspect to M. hyopneumoniae (Table 1).
The **group 1a**, with 15 animals, represents the group 1 after vaccination against erysipelas and pleuropneumonia. Following to the ELISA test, the results were: 15 positive to PRRS, 8 suspect to Circovirus and none to erysipelas (Table 2).
The **group 2**, with 30 animals of 65 days old, unvaccinated against PRRS, vaccinated against erysipelas with subunitar vaccine and against pleuropneumonia, were recorded 30 positive animals to PRRS, none to M. hyopneumoniae, 6 suspect to Circovirus and none to erysipelas (Table 3).
The **group 3**, with 20 animals of 70 days old, unvaccinated against PRRS and vaccinated against erysipelas, were recorded 20 positive to PRRS, 1 positive to M. hyopneumoniae, 5 suspect to Circovirus and none to erysipelas (Table 4).
The **group 4**, with 20 animals of 70 days old, vaccinated against erysipelas and PRRS, presented 20 animals positive to PRRS, 2 positive to M. hyopneumoniae, 1 suspect to Circovirus and none to erysipelas (Table 5).
The **group 5**, with 20 animals of 65 days old, vaccinated against Circovirosis and M. hyopneumoniae, unvaccinated against erysipelas and PRRS, presented: 1positive animal to Circovirus and none positive to PRRS, Circovirus and erysipelas (Table 6).
The **group 5a**, with 30 animals of 83 days old represents the group 5 after vaccination against erysipelas by subunitar vaccine. Were obtained negative results to PRRS and Circovirus; 4 positive to M. hyopneumoniae and 1 suspect; 2 positive animals to erysipelas.

The **group 5b**, with 30 animals, represents the group 5 at the 91 days old. Were recorded 6 positive results to PRRS; 2 suspect to Circovirus; 1 suspect to M. hyopneumoniae; 5 positive to erysipelas.
The **group 6**, with 32 animals of 70 days old. The animals unvaccinated against erysipelas and PRRS, were grouped in 2 boxes as follows: the 1-st box with 16 animals (1-16) presented 1 animal suspect to M. hyopneumoniae and none to PRRS, Circovirus and erysipelas.
In the second box (17-32) was recorded 1 suspect animal to Circovirus and none to PRRS, M. hyopneumoniae and erysipelas (Table 9).
The **group 6a**, represents the group 6 after vaccination against erysipelas. The vaccination was applied using two vaccines from two different producers. In the 1-st box after vaccination with Eriromvac, 16 animals were positive to PRRS, 1 animal was suspect to Circovirus and the rest of animals were negative to M. hyopneumoniae and erysipelas; in the 2-nd box the animals were vaccinated with Bioveta vaccine and were recorded 16 positive animals to PRRS and negative to M. hyopneumoniae, 1 suspect to Circovirus and 4 positive to erysipelas (Table 10).

Table 1

Group 1. Age 65 days, weight 18 kg					
	Sampling date 06.03.2012	Immunisation: not vaccinated	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			49/50	0/50	0/50	
TOTAL suspect / tested			0/50	0/50	21/50	

Table 2

Group 1a. Sampling II, after erysipelas and pleuropneumonia vaccination on 08.03.2012						
	Sampling date 20.04.2012 (first sampling on 06.03.2012)	Immunisation: vaccinated for erysipelas and pleuropneumonia on 08.03.2012	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			15/15	0/15	0/15	0/15
TOTAL suspect / tested			0/15	0/15	8/15	0/15

Table 3

Group 2. Age 65 days, weight 40-45 kg					
	Sampling date 06.03.2012	Vaccinated for erysipelas and pleuropneumonia (subunit vaccine) on 02.02.2012 Not vaccinated for PRRS	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			30/30	0/30	0/30	0/30
TOTAL suspect / tested			0/30	0/30	6/30	0/30

Table 4

Group 3. Age 60 days					
	Sampling date 20.04.2012	Vaccinated for erysipelas on 15.03.2012. Not vaccinated for PRRS	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			20/20	1/20	0/20	0/20
TOTAL suspect / tested			0/20	0/20	5/20	0/20

Table 5

Group 4. Age 70 days					
	Sampling date 20.04.2012	Vaccinated for erysipelas and PRRS on 05.04.2012	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			20/20	2/20	0/20	0/20
TOTAL suspect / tested			0/20	0/20	1/20	0/20

Table 6

Group 5. Age 65 days						
	Sampling date 20.04.2012	Vaccinated for Circovirus and M. hyopneumoniae on 7 days of age. Not vaccinated for erysipelas and PRRS.	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			0/20	1/20	0/20	0/20

Table 7

Group 5a. Age 83 (65+18) days – sampling II					
Sampling date 07.05.2012	Vaccinated for M. hyopneumoniae and Circovirus on 24.03.2012. Vaccinated or erysipelas on 24.04.2012 (subunit vaccine).	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/30	4/30	0/30	2/30
TOTAL suspect / tested		0/30	1/30	0/30	0/30

Table 8

Group 5b. Age 91 (65+18+8) days – sampling III					
Sampling date 15.05.2012	Vaccinated for M. hyopneumoniae and Circovirus on 24.03.2012. Vaccinated or erysipelas on 24.04.2012 (subunit vaccine)	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		6/30	0/30	0/30	5/30
TOTAL suspect / tested		0/30	1/30	2/30	0/30

Table 9

Group: Lot 6. Age 70 days						
Sampling date 10.05.2012		Not vaccinated for erysipelas and PRRS	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		Box 1 (1-16)	0/16	1/16 (suspect)	0/16	0/16
		Box 2 (17-32)	0/16	0/16	1/16 (suspect)	0/16

Table 10

Group 6a. Age 98 days (70+28) – sampling II						
Sampling date 13.06.2012		Vaccinated for erysipelas on 15.05.2012 (Eryromvac box 1, Bioveta box 2)	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		Box 1 (1-16)	16/16	0/16	1/16 (suspect)	0/16
		Box 2 (17-32)	16/16	0/16	1/16 (suspect)	4/16

FARM B

The **group 1**, with 28 unvaccinated animals. Following to the serological tests were recorded 28 positive animals to PRRS, 5 positive to M. hyopneumoniae, 1 positive and 5 suspect animals to Circovirus (Table 11).

The **group 1a,** with 28 animals, represents the group 1 after vaccination against erysipelas. Were recorded 25 positive samples to PRRS, 7 suspect to Circovirus and all samples were negative to M. hyopneumoniae and erysipelas (Table 12).

The **group 2** with 30 unvaccinated animals. Were recorded 29 positive animals to PRRS; 6 positive and 4 suspect to M. hyopneumoniae; 1 positive and 6 suspect to Circovirus (Table 13).

The **group 2a,** with 22 animals, represents the group 2 after vaccination against erysipelas. Were recorded 22 animals positive to PRRS; 9 positive and 21 suspect to Circovirus, and all animals were negative to erysipelas (Table 14).

The **group 3**, with 30 vaccinated sows against E. coli and anaerobic germs, unvaccinated against erysipelas. In this group were recorded 27 positive animals to PRRS; 14 positive and 3 suspect to M. hyopneumoniae; 15 positive and 11 suspect to Circovirus (Table 15).

The **group 3a**, with 30 animals represents the group 3 after vaccination against erysipelas. Were detected 30 positive animals to PRRS; 13 positive and 3 suspect to M. hyopneumoniae; 5 positive and 10 suspect to Circovirus and 19 positive to erysipelas (Table 16).

Table 11

Group 1.Grower, age 37 days, F2C2/2					
Sampling date 08.03.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		28/30	5/30	1/30	
TOTAL suspect / tested		0/30	0/30	5/30	

Table 12

Group 1a. Grower, age 79 days (37+42) – sampling II					
Sampling date 19.04.2012	Vaccinated for erysipelas on 17.03.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		25/28	0/28	0/28	0/28
TOTAL suspecte / tested		0/28	0/28	7/28	0/28

Table 13

Group 2. Fattening, age 99 days F2C1/3					
Sampling date 08.03.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		29/30	6/30	1/30	
TOTAL suspect/ tested		0/30	4/30	6/30	

Table 14

Group 2a. Fattening, age 141 days (99+42) – sampling II					
Sampling date 19.04.2012	Vaccinated for erysipelas on 17.03.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		22/22	9/22	1/22	0/22
TOTAL suspect / tested		0/22	9/22	21/22	0/22

Table 15

Group 3. Sows F2C3/4					
Sampling date 08.03.2012	Vaccinated for E. coli and anaerobiosis. Not vaccinated for erysipelas.	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		27/30	14/30	15/30	
TOTAL suspect / tested		0/30	3/30	11/30	

Table 16

Group 3a. Sows - sampling II					
Sampling date 25.05.2012	Vaccinated for E. coli and anaerobiosis. Vaccinated for erysipelas on 23.04.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		30/30	13/30	5/30	19/30
TOTAL suspect / tested		0/30	3/30	10/30	0/30

HOUSEHOLD FARMS

Group 1, with 20 completely unvaccinated animals, of 4 months old. Were recorded 5 suspect animals to Circovirus, the rest of animals being negative to the other diseases including erysipelas (Table 17).
Group 1a, with 20 animals of 5,5 months old, vaccinated against erysipelas with Eryromvac vaccine. All animals have been found negative to PRRS

and M. hyopneumoniae; 3 suspect animals to Circovirus and 12 positive to erysipelas (Table 18).
The **group 2,** with 20 unvaccinated animals of 6 months old. All animals were negative to PRRS and M. hyopneumoniae; 5 animals were suspect to Circovirus and 4 animals were positive to erysipelas.
The **group 2a,** with 20 animals, represents the group 2 of 7.5 months old, after vaccination against erysipelas. All animals were found negative to PRRS and M. hyopneumoniae, 5 animals were suspect to Circovirus and 11 animals were positive to erysipelas (Table 20).
Lotul 3, compus din 10 animale, vârsta de 1 an, total nevaccinate. Au fost depistate toate animalele negative la PRRS și M.hyopneumoniae. 2 dubioase la Circovirus și 5 pozitive la Rujet (Table 21).

Table 17

Group 1. Age 4 months, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	0/20
TOTAL suspect / tested		0/20	0/20	5/20	0/20

Table 18

Group 1a. Age 5.5 months, household farm – sampling II					
Sampling date 07.06.2012	Vaccinated for erysipelas (Eryromvac) on 11.05.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	12/20
TOTAL suspect / tested		0/20	0/20	3/20	0/20

Table 19

Group 2. Age 6 months, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	4/20
TOTAL suspect / tested		0/20	0/20	5/20	0/20

Table 20

Group 2a. Age 7.5 months, household farm– sampling II					
	Sampling date 07.06.2012	Vaccinated for erysipelas (Eryromvac) on 11.05.2012	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			0/20	0/20	0/20	11/20
TOTAL suspect / tested			0/20	0/20	5/20	0/20

Table 21

Group 3. Age 1 year, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/10	0/10	0/20	5/10
TOTAL suspect / tested		0/10	0/10	2/20	0/10

Percentage representation of the antibody titer induced by the immune-suppressor germs studied (PRRS, M.hyopneumoniae, Circovirus) and the erysipelas antibody level following to the vaccination against erysipelas are presented by the histograms in figures 1, 2 and 3.

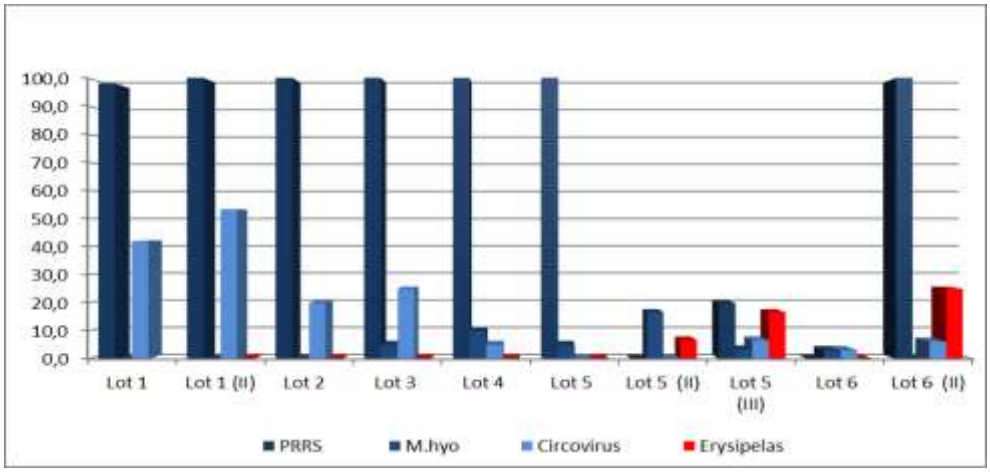

Figure 1. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm A.

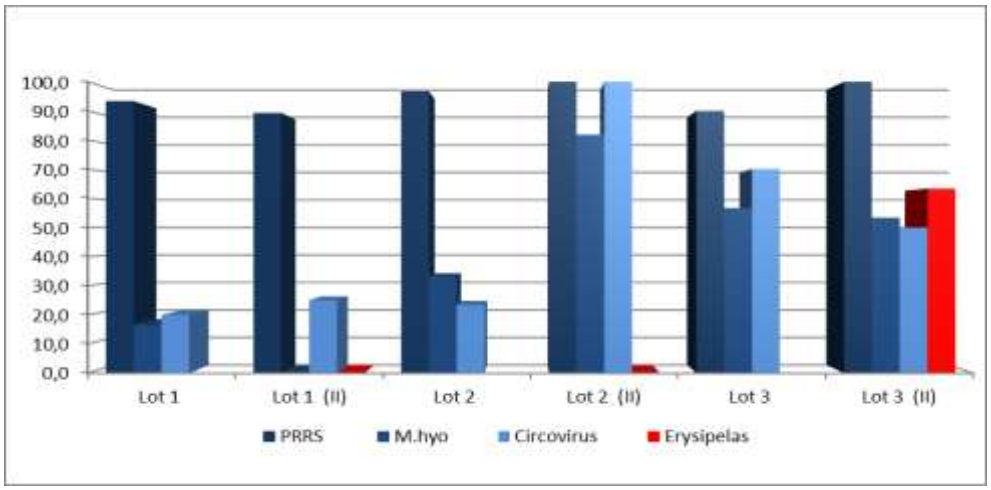

Figure 2. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm B.

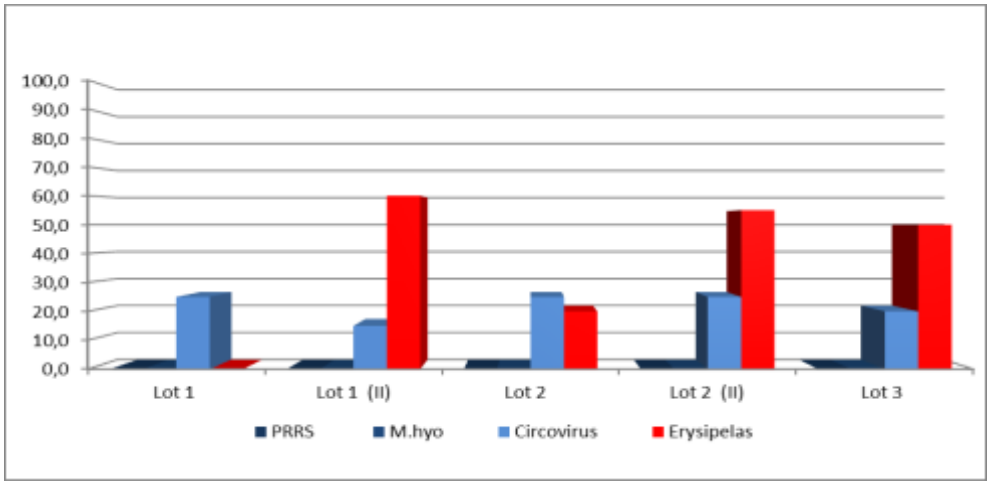

Figure 3. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm C.

Concomitantly with isolation of the proposed germs (PRRS virus, circovirus or Mycoplasma hyopneumoniae) we had in intention to determine the associated infections, produced by the pathogenic microbial flora or with

pathogenic potential, infections which may have an influence on the immune status of animals.
By bacteriological examinations were isolated bacterial association germs, pathogens or potential pathogens. Have been isolated from pigs, the following bacteria with pathological significance : *Mannheimia (Pasteurella) haemolytica* (1 strain), *Pasteurella multocida* (2 strains), *Pseudomonas aeruginosa* (2 strains), *Streptococcus spp.*(3 strains), *Klebsiella pneumoniae* (1 strain*), Escherichia coli* (12 strains).

CONCLUSIONS

Preliminary results indicated a link between the existence of the studied microbial agents and the immune response after vaccination. Thus, following anti-erysipelas vaccination applied in three pig units, with different immune status and growth conditions, the seroconversion was negative in high proportion, regardless of the type of vaccine used, alive or subunit.
Investigations show the presence of Circovirus and PRRS virus and also M. hyopneumoniae in pigs populations from intensive farming systems which practiced animals import. Clinically healthy animals, suggest a carrier status, clinical signs being dependent on the sanitary status of the farm and the presence of other pathogens.
The study shows that both microbial agents studied (PRRS, Circovirus, Mycoplasma hyopneumoniae) and the germs of association have a negative action on the effectiveness of immunoprophylaxis programs applied in breeding pigs.

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