Epidemiological Investigation of Pseudorabies in Greece

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Abstract Pseudorabies is an acute, frequently fatal disease that mainly affects pigs and incidentally other animals. Although pseudorabies virus (PRV) has been eradicated from many European countries, it is still endemic in East and Southeast parts of Europe. Greece belongs to the countries where the disease is enzootic. In this study, we investigated the presence of PRV in Greek farms. For that reason, 42 pig farms were selected from the entire Greek territory. Blood samples from different age groups had been collected from each farm and were tested by ELISA for the presence of antibodies against wild strains of PRV. The results of our study showed that 28.6% of the selected farms were positive for the presence of antibodies against wild-type strains of PRV and that factors such as the non-implementation of biosafety measures and the high density of pig farms in an area may affect the probability of a farm to become PRV positive. This study provided some useful information with regard to the presence of PRV in the domestic pigs in Greece. This information may assist in designing and implementing measures in order to control and eradicate the disease from the domestic pigs in Greece.

Keywords Pseudorabies · Epidemiology · Pigs · Eradication

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1 Introduction

Aujeszky's disease or pseudorabies is an acute, frequently fatal disease that mainly affects pigs and incidentally other domestic and wild animals. The term "pseudorabies" was used as a result of the disease's clinical resemblance to rabies. The disease was first described in 1813 in cattle, which were showing extreme pruritus. Aladar Aujeszky was the Hungarian veterinarian who first described and reproduced the disease in 1902, providing evidence that the etiologic agent was filterable (e.g. not a bacterium but a virus) (Mettenleiter et al. 2012). PRV has been classified in the genus *Varicellovirus,* subfamily *Alphaherpesvirinae* and family *Herpesviridae.* The virus particles have the typical architecture of a herpes virion (Mettenleiter 2000). The various strains of PRV differ in their infectivity and virulence in pigs as well as in their ability to be shed during infection. Those differences are associated with identified differences in their genome (Nauwynck et al. 2007). PRV pathogenesis depends on the age of the pig, the dose, the route of the inoculation and the virus strain. More specifically, the development and severity of the clinical signs of the disease diminish with increasing age. Neuronal signs are severe in pigs prior to weaning while fatteners are relatively resistant to nervous disease, showing mainly respiratory symptoms. In boars and sows, PRV infection is characterized by symptoms from respiratory and reproductive systems (Mettenleiter 2000; Papageorgiou et al. 2011a). Although swine is the only natural host of the virus, PRV can also infect a large number of species including cattle, sheep, goats, cats, dogs and wildlife (Pensaert and Kluge 1989; Banks et al. 1999; Bitsch and Munch 1971; Glass et al. 1994). There are no published data for PRV infection in humans (Tischer and Osterrieder 2010).

PRV is spread all over the world, particularly in regions with dense pig populations, including Europe, Asia and America. In Europe, PRV has been eradicated in Germany, Cyprus, Austria, Sweden, The Netherlands, Denmark, Czech Republic, Finland, France, Hungary, Luxemburg, Belgium, Switzerland, Slovakia and the UK as a result of the implementation of eradication programmes, but it is still endemic in East and Southeast of Europe (Hahn et al. 2010). PRV has also been eradicated from Canada, New Zealand and the USA (MacDiarmid 2000). More specifically, the USA has been classified as free of the disease since 2007. Although PRV has been eradicated from many countries throughout the world, the virus is still endemic in the populations of wild boar (Meng et al. 2009; Millicevic et al. 2016; Verpoest et al. 2014; Muller et al. 2011). Therefore, these populations should be considered as potential PRV source of infection for domestic pigs. In countries that are free of PRV, vaccination is prohibited.

Greece belongs to the countries where the disease is still enzootic. Up to 1973, only sporadic cases of Pseudorabies were diagnosed in bovine and sheep populations. According to an old serological study in 1969 (Papatsas et al. 1995), 20.8% of the collected blood samples of domestic pigs from several regions of Greece were positive to the presence of antibodies against PRV. But at that time, there was no serious and organized pig farming in Greece. In addition, although two recent Greek studies (Touloudi et al. 2015; Marinou et al. 2015) evidence the presence of PRV in 32% to 35% in wild boars, there is no recent data regarding the presence of PRV in the population of Greek domestic pigs. Here, we conducted an epidemiological study in order to investigate the presence of PRV in the Greek pig farms.

2 Materials and Methods

2.1 *Characteristics of the Study Population*

The study was carried out in Greece from October 2010 to October 2011. Greece produces up to 40% of the pork consumed within the country. The size of the country is approximately 132,000 km^2, 75% of which is covered by mountains. The pig population of the country is estimated to be around 1.2 million, with at least 88% of this population produced in 315 individually owned closed one-site system farrow-to-finish (FTF) farms of sizes larger than 100 sows. The majority of these farms are located in continental Greece and Crete, belong to individual persons, apply intensive indoor housing and have their own feed mill. As such farms reflect the commercial pig industry, the current study included FTF herds larger than 100 sows.

2.2 *Selection of Herds*

Forty-two (42) farrow-to-finish (FTF) pig herds were selected from the entire Greek territory at random, based on geographical criteria, in order to obtain representative data from the population herds. The sample represented more than 10% of the FTF farms. The herds were divided according to their size in two categories: up to 300 sows (small farms) and larger than 301 sows (large farms) (Table 1). As a first step, the selected farmers were contacted by phone in order to introduce the purpose of the study. Consequently, an appointment was arranged for the team in order to visit the farms, to collect data about the herds and the area where these holdings were located and to obtain the samples.

2.3 *Collection of Herd Information*

The data were obtained through face-to-face interviews of the owners of the selected farms and the following inspection of the pig units. The obtained information pertained to:

Table 1 Characteristics of commercial pig herds in Greece (>100 sows) and herd sampling for the study

Territory	Area (km^2)	Density (# of farms/ 1000 km^2)	Number of farms sampled /number of farms in territory *Herd-size category*		
			Small	**Large**	**Total**
East Macedonia and Thrace	*19,000*	*1.4*	*4/16*	*1/10*	*5/26 (19.2)*
Central and West Macedonia	*25,000*	*1.1*	*4/18*	*5/11*	*9/29 (31.0)*
Thessalia	*14,000*	*6.9*	*4/81*	*3/16*	*7/97 (7.2)*
Epirus and West Sterea Hellas	*15,000*	*6.1*	*2/66*	*6/24*	*8/90 (8.9)*
East Sterea Hellas	*20,000*	*1.9*	*7/21*	*4/15*	*12/36 (30.6)*
Peloponnesos and Crete	*30,000*	*0.9*	*1/25*	*1/12*	*2/37 (5.4)*
Total	*123,000*	*2.4*	*22/227 (9.7%)*	*20/88 (22.7%)*	*42/315 (13.3%)*

- Herd size, e.g. the number of sows on the premises. Farms with less than or equal to 300 sows were considered as small, while those with more than 300 sows as large.
- Pig herd area density, e.g. less dense (<20 farms per 1000 km^2) or more dense ($\geq$20 farms per 1000 km^2) areas.
- Direct distance from the closest pig farm, e.g. short (<6 km) or longer ($\geq$6 km) distance. All the selected farms did not include more than one neighbouring pig farm within 6 km radius. No other pigs, except breeding animals, and no semen were entering the farm.
- Purchase (or not) of breeding animals (gilts or/and boars) from genetic companies. No other pigs – except breeding animals – and no semen were entering the farm. In the herds that did not purchase breeders, gilts were produced within the farm by a grandparent stock of breeders.
- Practising (or not) of at least monthly quarantine in a distant building used exclusively for the newly purchased breeding animals.
- Practising (or not) of certain hygienic/biosecurity measures at farm. More specifically, (a) regular cleaning followed by disinfection and a stand-empty period of a minimum of 3 days between two production groups; (b) prevention of entry of lorries carrying pigs, feed or manure; (c) fence and barrier at entrance; and (d) prevention of entry of visitors, presence and use of a sanitary room to change clothes and use of unit protective clothing (but not always downtime and showering applicable).
- Practising (or not) of all-in/all-out (AIAO) flow in all production stages. A compartment was defined as a subdivision of a building with its own ventilation system. AIAO was considered to take place if the compartment (with its own

ventilation system) was filled up the same day, was emptied in one or two times and when a complete depopulation had taken place prior to restocking.
- Practising (or not) of vaccination for PRV.
- Presence (or not) of substantial economic problems in the farm that frequently interfere with routine management. Farmer's psychology and motivation for careful and extra work, as well as undisrupted supply of feed ingredient, medication and vaccines, were the immediate consequences of these economical problems.
- Presence (or not) of certain systemic clinical manifestations at the time of sampling, such as mortality, nervous, respiratory, gastrointestinal or/and reproductive health problems in one or more production stages in the farm.
- Production stage at which important clinical manifestations were present (or not) at the time of sampling (neonatal, nursery, grower and finishing stage).

2.4 Sampling and Laboratory Testing

A minimum of 8 blood samples from each out of 5 different age groups (i.e. 6-, 8-, 10-, 12- and 22-week old pigs) had been collected from each farm (in total 1723 blood samples). The blood samples for each age group were collected from pigs of different pens and, ideally, of different rooms. Sera were individually tested by anti-PRV-gB ELISA (IDEXX Laboratories, Westbrook, ME) for the presence of antibodies against the PRV and by anti-PRV-gE ELISA (IDEXX Laboratories, Westbrook, ME) for the differentiation of antibodies against the wild strains of PRV.

2.5 Statistical Analysis

Apparent prevalence of PRV-infected farms was estimated as the proportion of farms rearing at least one pig presenting antibodies against PRV, while its corresponding confidence interval (95% CI) was calculated around the estimated apparent prevalence using the exact method. Multiple correspondence analysis (MCA) has been used in order to investigate most profound interactions among several variables, particularly categorical, in order to localize dominant and more substantial trend in their structure. No prior precondition (e.g. distribution that should follow the data or models that we assume that apply to the population) is needed (Moschidis 2009, 2015; Moschidis and Chadjipadelis 2017).

The association of the herd and neighbourhood characteristics of the farms (predictors) with PRV status was investigated through the application of invariable logistic regression models with robust standard errors. Subsequently, a multivariable logistic regression model was built using as predictors the variables that presented a strong invariable association with PRV status (p-value <0.10). Multicollinearity between these latter predictors was estimated, selecting variables that exhibit tolerance greater than 0.4, following Lambert et al. (2012) methodological approach. The Hosmer-Lemeshow goodness of fit was estimated for the assessment of the final multivariable logistic regression model. The SPSS software (Version 22.0) was used.

3 Results

The sample used in this study represented 13.3% of the total commercial FTF pig herds larger than 100 sows in the country, including almost 10% of the small farms and 23% of the large farms. The characteristics of the total sampled herds are presented in Table 2. According to the data, 83.3% of the selected farms were located in areas with fewer than 20 farms (low herd density areas), 66.7% were located to close distance (<6 km) from other farms, 69.0% were purchasing gilts or/and boars from an outside source, 78.0% were not applying quarantine, 66.7% were suffering from substantial economic problems and 61.9% and 52.4% were suffering from respiratory and reproductive problems, respectively, while the main health problems in most of these herds were observed during nursery and grower stage (69.0% and 61.9% of total herds, respectively). Moreover, of the total number of selected herds (42), in 35 farms (83.3%), pigs were vaccinated against PRV.

Table 3 shows wild-type PRV-positive farms throughout the Greek territory. More specifically, the exposure of the farms to wild-type PRV was 28.6% with most of the positive holdings located in the region of East Macedonia and Thrace and Central and West Macedonia (Fig. 1).

The proportion of positive PRV farms for the level of each predictor is indicated in Table 2. More specifically, 53.8% of the PRV-positive herds were located in farm low-density areas, 91.7% had a direct distance of less than 6 km from other herds, 83.3% were purchasing gilts or/and boars from an outside source, 100% were not applying quarantine during the entrance of newly purchased pigs, 69.2% were suffering from substantial economic problems and 83.3% and 58.3% were suffering from respiratory and reproductive problems, respectively, while the main health problems in 83.3% and 91.7% of these herds were observed during nursery and grower stage, respectively.

The results of MCA are presented in Tables 4 and 5. Factorial axes F1 and F2 interpret 44.32% and 16.08% of inertia, respectively (e.g. both 60.4% of inertia) (Fig. 2). Points with CTR larger than 20 (1000/48) were preserved in axes.

In the first tendency (F1 axis) (Table 4), two roughly opposite groups of farms and characteristics were observed (denoted by opposite signs): (A) a group of farms that were practising basic hygienic measures and quarantine and did not suffer from major economic problems. These farms were not purchasing breeding animals from other farms. In these farms, respiratory, reproductive and general health problems were not issues, while nursery, growing and finishing phases were not affected. (B) A group of farms with PRV that were mostly suffering from economic problems and did not practise basic hygienic measures. In these farms, growing and finishing

Table 2 Characteristics of the sampled breeding farms in Greece (42 farms) and descriptive statistics for predictors tested for association with PRV-positive status

Predictors	Category	Farms (number)	*(%)*	Number of positive sites (%)	Characteristic within positive herds (%)
Size (no sows)	<300	22	*52.4*	6 (27.3)	50.0
	≥300	20	*47.6*	6 (30.0)	
Density (farms/1000 km^2)	<20	35	*83.3*	7 (20)	53.8
	≥20	7	*16.7*	5 (71.4)	
Distance (km)	<6	28	*66.7*	11 (39.3)	91.7
	≥6	14	*33.3*	1 (7.1)	
Gilt purchase	No	13	*31.0*	2 (15.4)	
	Yes	29	*69.0*	10 (34.5)	83.3
Quarantine	No	32	*78.0*	12 (37.5)	100.0
	Yes	9	*22.0*	0 (0.0)	
Biosecurity measures	No	19	*45.2*	10 (52.6)	83.3
	Yes	23	*54.8*	2 (8.7)	
AIAO	No	18	*43.9*	8 (44.4)	66.7
	Yes	23	*56.1*	4 (17.4)	
PRV vaccination	No	7	*16.7*	3 (42.9)	
	Yes	35	*83.3*	9 (25.7)	75.0
Economic problems	No	14	*33.3*	3 (21.4)	
	Yes	28	*66.7*	9 (32.1)	69.2
Mortality	No	32	*76.2*	6 (18.8)	
	Yes	10	*23.8*	6 (60.0)	50.0
Nervous signs	No	36	*85.7*	9 (25.0)	
	Yes	6	*14.3*	3 (50.0)	25.0
Respiratory signs	No	16	*38.1*	2 (12.5)	
	Yes	26	*61.9*	10 (38.5)	83.3
Gastrointestinal signs	No	27	*64.3*	7 (25.9)	
	Yes	15	*35.7*	5 (33.3)	41.7
Reproductive signs	No	20	*47.6*	5 (25.0)	
	Yes	22	*52.4*	7 (31.8)	58.3
Neonatal stage problems	No	39	*92.9*	9 (23.1)	
	Yes	3	*7.1*	2 (66.7)	16.7
Nursery stage problems	No	13	*31.0*	2 (15.4)	
	Yes	29	*69.0*	10 (34.5)	83.3
Grower stage problems	No	16	*38.1*	1 (6.3)	
	Yes	26	*61.9*	11 (42.3)	91.7
Finisher stage problems	No	29	*69.0*	5 (17.2)	
	Yes	13	*31.0*	7 (53.8)	58.3

Table 3 Exposure of Greek farms to wild-type PRV as detected by ELISA

	PRV gE-ELISA antibody-positive farms /number of farms sampled (%) *Herd-size category*		
Territory	**Small**	**Large**	**Total**
East Macedonia and Thrace	1/4	1/1	2/5 (40.0%)
Central and West Macedonia	2/4	2/5	4/9 (44.4%)
Thessalia	1/4	1/3	2/7 (28.6%)
Epirus and West Sterea Hellas	1/2	2/6	3/8 (37.5%)
East Sterea Hellas	1/7	0/4	1/11 (9.1%)
Peloponnesos and Crete	0/1	0/1	0/2 (0.0%)
Total	**6/22 (31.6%)**	**6/20 (27.3%)**	**12/42 (28.6%)**

Fig. 1 The stars on the map indicate the location of the selected farms on the Greek territory. The red and the green stars indicate the positive and negative farms, respectively, to the presence of antibodies against the wild-type strains of PRV

Table 4 Multiple correspondence analysis (MCA) results – F1 axis

Variable	ID	#F1	COR	CTR
Absence of general health problems	**C11**	638	802	73
Absence of problems in grower pigs	**D31**	465	796	66
Absence of respiratory symptoms	**C41**	438	771	58
Absence of major economic problems	**B51**	420	672	47
Animal quarantine during entrance	**B72**	487	564	38
Application of basic hygienic measures	**B82**	260	646	29
Absence of problems in nursery (weaned) pigs	**D21**	336	479	28
No purchase of breeding animal from outside	**B61**	332	493	27
Absence of reproductive symptom	**C61**	264	542	26
Absence of problems in finisher pigs	**D41**	192	688	20
Presence of major economic problems	**B52**	−211	672	23
Presence of general health problems	**C12**	−200	802	24
Presence of reproductive symptom	**C62**	−241	542	24
Presence of increased mortality	**C22**	−441	517	34
No application of basic hygienic measures	**B81**	−316	646	35
Presence of respiratory symptoms	**C42**	−270	771	36
Problems in grower pigs	**D32**	−287	796	41
PRV-infected	**A11**	−456	658	44
Problems in finisher pigs	**D42**	−430	688	46

CTR contributions, *COR* projections

Table 5 Multiple correspondence analysis (MCA) results – F2 axis

Variable	ID	#F2	COR	CTR
Problems in neonates	**D12**	679	398	68
No vaccination of sows for PRV	**B101**	426	454	63
No application of AIAO system	**B91**	219	364	47
East Sterea Hellas	**B15**	259	296	36
Small farms	**B21**	149	240	25
Presence of gastroenteric symptoms	**C52**	−186	212	27
Large farms	**B22**	−165	240	28
Application of AIAO flow system	**B92**	−182	364	39
Vaccination of pigs for PRV	**B112**	−424	391	62
High-density area	**B32**	−496	467	85
Epirus and West Sterea Hellas	**B14**	−578	801	132

CTR contributions, *COR* projections

pigs were mostly affected, and increased mortality, respiratory, reproductive and general health problems were mostly evident.

In the second tendency (F2 axis) (Table 5), two groups of farms and characteristics had been formed: (A) A group of small farms, mostly located in East Sterea Hellas, in which vaccination of sows against PRV and AIAO was not practised and

Total inertia 0.12536

Axis	Inertia	%Interpretation	Sum	Histogram of characteristic roots.
1	0,0555556	44,32	44,32	|***
2	0,0201522	16,08	60,39	|***************
3	0,0137212	10,95	71,34	|**********
4	0,0073600	5,87	77,21	|******
5	0,0068333	5,45	82,66	|*****
6	0,0055255	4,41	87,07	|****
7	0,0032446	2,59	89,66	|***
8	0,0024631	1,96	91,62	|**
9	0,0023905	1,91	93,53	|**
10	0,0019312	1,54	95,07	|**
11	0,0012693	1,01	96,08	|*
12	0,0010156	0,81	96,89	|*

Fig. 2 Table of inertia

neonates were mostly affected. (B) A group of large farms located in high-density areas (e.g. Epirus and West Sterea Hellas), practising vaccination of fatteners against PRV as well as AIAO system, in which gastroenteric symptoms were most often seen.

In logistic regression models, the variable that expresses the quarantine measures had to be excluded from the regression analysis due to absence of variation in the values for positive sites, i.e. the dependent variable (presence of virus) didn't vary within this variable. After excluding these variables, 17 predictors were used in the logistic regression in order to investigate their association with PRV status. The predictors that appear to have an invariable association with PRV status at significance level of 0.10 (p-value $\leq$0.10) are presented in Table 6. Results indicate that 9 out of the 15 predictors were strongly related to PRRSV status in the farms; however, two variables, namely, those that express respiratory health issues and health issues at grower stage, were excluded from the multivariable logistic model due to multicollinearity.

According to the results (Table 7), it appears that factors such as "pig herd area density" and "hygienic/biosecurity measures" play a key role in the probability of a farm to become PRV positive. More specifically, farms, which were located in low-density areas and were applying hygienic/biosecurity measures, had a predicted probability of being positive for PRV of 1.97%. However, their probability was increased to 26.7% when farms were located in low-density areas but were not applying hygienic/biosecurity measures. Farms that were located in high-density areas, applying hygienic/biosecurity measures, had a predicted probability of being positive for PRV of 30.8%. Finally, the probability of being positive for PRV was increased to 88.9% when farms were located in high-density areas and were not applying hygienic/biosecurity measures.

Table 6 Predictors associated (p-value ≤0.10) with PRV-positive status using invariable logistic regression assuming robust SE

Predictors	Odds ratio	95% CI	P-value
Farm size (≥ 300 sows per farm)	0.255	0.06–1.16	0.076
Density (≥farms/1000 km^2)	4.500	0.81–24.95	0.085
Distance from closest farm ≥6 km	0.119	0.01–1.07	0.057
Biosecurity measures	0.086	0.02–0.48	0.005
AIAO	0.263	0.06–1.11	0.069
Mortality	6.500	1.36–31.06	0.019
Grower stage problems	10.999	1.23–98.80	0.032
Finisher stage problems	5.600	1.28–24.42	0.022

Table 7 Predictors associated (p-value ≤0.05) with PRV-positive status using multivariable logistic regression

Predictors	b	SE(b)	Odds ratio	95% CI	Wald test	P-value
Intercept	−1.010					
Density	3.097	1.798	22.14	−0.42 – 6.62	1.72	0.085
Biosecurity measures	−2.894	1.174	0.06	−5.19 – −0.59	−2.47	0.014

Hosmer-Lemeshow goodness-of-fit test, p-value = 0.35

4 Discussion

Up to 1973, only sporadic cases of PRV in sheep, bovine and mink had been diagnosed in Greece. The first clinical report, followed by virus isolation of PRV in pigs, was on May 1974. Two more clinical cases with high mortality of suckling piglets had been reported on January 1976 and February 1977. In 1983, the import of breeding animals from other European countries caused outbreaks of the disease in the whole country (Papadopoulos 1989; Papadopoulos et al. 1996; Papatsas et al. 1995). According to the previous published study of 1969, antibodies were found in 20.8% of the tested serum samples. It is necessary to point out that that study refers to swine blood serum samples which were sporadically tested before the "industrialization" of pig farming and before the onset of vaccination programmes (the vaccinations for PRV in Greece started in the mid-1980s but never applied in a systematic way). In the present study, the majority of the positive farms (75%) was practising a vaccination scheme against PRV. The latter finding indicates that vaccination alone is not sufficient to eradicate the disease, unless it is accompanied by other measures such as the removal of the animals, which are found positive to the presence of antibodies against PRV. The aim of vaccination as a part of an eradication programme is not only to induce clinical protection but also to stop the transmission of the virus within and between herds by inducing herd immunity. Both attenuated and inactivated vaccines can be induced (Kritas 1994; Papageorgiou et al. 2011b). The development of marker vaccines and the use of diagnostic tests (differential ELISA) can play an important role in the eradication and control campaigns, as it was determined in the PRV eradication programme in the USA (Foley and Hill 2005; Papageorgiou et al. 2011b).

The proportion of positive PRV farms for the level of each predictor is indicated in Table 2. Of the PRV-positive herds, 91.7% had a direct distance of less than 6 km from other herds, 83.3% were purchasing breeding animals (gilts or/and boars) from sources outside the farm, 83.3% were not practising certain biosecurity measures and 100% of the PRV-infected farms did not apply quarantine for the newly purchased breeding animals! The analysis of the data also showed that 69.2% of the PRV-infected farms were facing substantial economic problems. The factor "economic problems" had been added to the questionnaire as a result of the crisis which affects the Greek economy in the last 8 years. It had been addressed to the owner but also to key personnel in a direct or indirect way. The reason was to include or detect purposefully or latently missing "gaps" in routine management, biosecurity or hygiene. For example, medication and vaccines may have been properly purchased in the farm, but their application was not correct due to underpaid personnel or not supervised closely due to bad psychology of the farmer. More specifically, among other things, the crisis led some farmers to abandon vaccination against PRV. It is important to keep in mind that although vaccination suppresses the manifestation of typical clinical signs of the disease, it doesn't eliminate the virus. One of the main characteristics of herpes viruses is latency. Latency is specified as a condition in which infectious virus is not produced, although viral DNA persists. For an unknown reason, most probably associated with stress factors, the virus is reactivated and subsequently may "come up" in the population. That was the reason for the re-emergence of the virus in many farms.

By using MCA, all variables can be simultaneously included and can reveal their most intense interactions, as well as dominant tendencies. Interpretation of the first axis shows that farms lacking basic hygienic measures suffer from major health problems (e.g. mortality, respiratory, reproductive and general health problems) particularly during fattening period. This is something expected as it is widely known that biosecurity measures prevent the entrance and circulation of pathogens within a farm. Apparently this is the reason why PRV was presenting such farms, where it may well contribute to all described symptomatology. As no detection of other pathogens had been attempted at present, it is not known whether the same applies for these as well, but it would be interesting to investigate it in the future by this analysis. Major economic problems were also associated with this group of farms, but it was not possible to distinguish whether they represented the result or the cause of the health problems.

The interpretation of second axis had added some more information to those derived by first axis. Thus, observations in small farms show that sow vaccination against PRV and application of AIAO flow system may be necessary to secure good health in neonates. In addition, observations in large farms show that fattener's vaccination against PRV and application of AIAO flow system may reduce PRV-associated symptoms (gastrointestinal symptoms are not considered typical for PRV) at least in high-density areas. It is known that specific maternal and active immunity may sufficiently protect neonates and fatteners, respectively, against PRV symptoms (Kritas et al. 1997; Papageorgiou et al. 2011b). The fact that increased mortality and neurological symptoms had not been observed in neonatal pigs and respiratory and reproductive clinical signs had not been observed in older pigs of these farms confirms the general good efficacy of vaccinations against PRV.

Furthermore, the results of the multivariable logistic regression analysis had end up with a quantitation of the correlation of two variables that are "pig herd area density" and "hygienic/biosecurity measures". Therefore, the probability of being PRV-infected can vary from 2% for a "clean farm" of a low herd density area to 89% for a "dirty farm" of a high herd density area. Although PRV is transmitted primarily between swine through nose-to-nose contact, under favourable conditions, the virus may spread by aerosols (Vannier 1988; Christensen et al. 1990). Thus, it is obvious that the higher the density of pig farms of an area, the more likely is that a farm will become positive for PRV. Factors such as movement of wild and domestic animals should also play a role in the spread of PRV in high-density areas.

This study provided some useful information with regard to the presence of PRV in the domestic pigs in Greece. In addition, the statistical analysis of the collected data shed light to the correlation of PRV-infected pig farms to factors such as pig area density and the application of certain biosecurity/hygienic measures. The ultimate objective of pseudorabies control is its eradication. Several PRV control and eradication programmes have been implemented in Europe and the USA (Andersson et al. 1997; Bech-Nielsen et al. 1995; Muller et al. 2003; Vannier 1988). Compared to other several European countries, which had already eradicated PRV, Greece has many important advantages (Papadopoulos et al. 1996):

- The low density of the pig population (7 pigs/km2 in Greece, when in Holland it is 400 pigs/km2, in Belgium 230 pigs/km2, in Germany 73 pigs/km2, in Italy, Portugal, Spain and France between 20 and 30 pigs/km2).
- The type of the units is principally farrow-to-finish having their own feed mill. Thus, entrance of virus in the farms can be better prevented when compared to the fattening type of units.
- As a country that imports most of its breeding stock, a PRV-free status of animals can be required from breeder countries.
- Vaccinations with live or inactivated gE-vaccines are regularly applied in the majority of the organized farms.

In the case that farmers wish to quit PRV vaccination, this should be done not based on clinical or post-mortem findings but on intense laboratory testing of the current and incoming stock. A qualified herd health management specialist on infectiology should direct such procedures plus all appropriate additional measures.

In conclusion, this study provides new information regarding the presence of PRV in Greek pig farms. More specifically, it is the first well-organized scheduled research for the epidemiological study of pseudorabies, a disease which causes serious economic problems in the Greek pig industry. The use of the obtained information may assist in the designation and implementation of measures in order to control and eradicate the disease from Greece.

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