Epidemiological Investigation of Pseudorabies in Greece

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Abstract. Pseudorabies, is an acute, frequently fatal disease, that mainly affects pigs. 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.

Keywords: pseudorabies, epidemiology, pigs, eradication

1 Introduction

Aujeszky's Disease or Pseudorabies, is an acute, frequently fatal disease, that primary 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. 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 is spread all over the world, in parts of Europe, Southeast 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 UK as a result of the implementation of eradication programs, 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 USA (MacDiarmid, 2000). 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). Therefore, these populations should be considered as potential PRV

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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. According to an old serological study in 1969 (Papatsas et al., 1995), 20.8% of the collected samples of domestic pigs from several regions of Greece were positive to 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

The study was carried out in Greece from October 2010 to October 2011. 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 current study included FTF herds larger than 100 sows, as that kind of farms most likely reflect the commercial pig industry of the country. The sample represented more than 10% of the FTF farms. The data regarding the characteristics of the selected herds are presented in table 1.

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

Territory	**Area (km^2)**	**Density (# farms/ 1000 km^2)**	**Number of farms sampled /Number of farms in territory Herd-size category**		
			Small	**Large**	**Total**
East Macedonia and Thraki	*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)*
Epiros 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 & Crete	*30,000*	*0.9*	*1/25*	*1/12*	*2/37 (5.4)*
TOTAL	*123,000*	*2.4*	*22/227*	*20/88*	*42/315*
			(9.7%)	*(22.7%)*	*(13.3%)*

The owners or the veterinarians of the selected farms, were contacted in order to obtain information about the farms and the characteristics of the area where these holdings were located. The obtained information pertained to:

- 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 1,000 sq km) or more dense (≥20 farms per 1,000 km^2) areas.
- direct distance from the closest pig farm, e.g. short (<6 km) or longer (≥6 km) distance.
- purchase (or not) of breeding animals (gilts or/and boars) from genetic companies.
- practicing (or not) of at least monthly quarantine in distant building used exclusively for the newly purchased breeding animals.
- practicing (or not) of certain hygienic/ biosecurity measures at farm.
- practicing (or not) of all-in, all-out (AIAO) flow in all production stages.
- practicing (or not) of vaccination of sows for PRV.
- presence (or not) of substantial economic problems in the farm that frequently interfere with routine management.
- presence (or not) of certain systemic clinical manifestations at the time of sampling.
- production stage at which important clinical manifestations were present (or not) at the time of sampling (neonatal, nursery, grower and finishing stage).

A minimum of 8 blood samples from each out of five different age groups (i.e. 6-, 8-, 10-, 12- and 22-week old pigs) had been collected from each farm (e.g. 40 samples per farm). 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.

Apparent prevalence of PRV infected farms was estimated as the proportion of farms rearing at least one pig presenting wild-type PRV antibodies. The association of the herd and neighborhood characteristics of the farms (predictors) with PRV status was investigated through the application of univariable logistic regression models with robust standard errors. The SPSS software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used. Multiple Correspondence Analysis (MCA) was also used to investigate the interaction and interrelations among all elements, aiming at the identification of the dominant and most substantial tendency in their structure.

3 Results – Discussion

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, Thrace and central-west Macedonia (table 2).

Table 2. Exposure of Greek farms to wild-type PRV as detected by ELISA

	ELISA- gE PRV antibody positive farms /Number of farms sampled (%) Herd-size category		
Territory	**Small**	**Territory**	**Total**
East Macedonia and Thrace	1/3	1/2	2/5 (40,0%)
Central and West Macedonia	2/3	2/6	4/9 (44,4%)
Thessaly	1/3	1/4	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%)
Peloponnese & Crete	0/1	0/1	0/2 (0,0%)
TOTAL	**6/19 (31,6%)**	**6/22 (27,3%)**	**12/42 (28,6%)**

In 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 tested before the «industrialization» of pig farming and before the onset of vaccination programs (the vaccinations for PRV in Greece started in the mid 80's), whereas in the present study, the majority of the farms (75%) is practicing 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 proportion of positive PRV farms for the level of each predictor is indicated in table 3. The analysis of the data presented in table 3 showed that 83.3% of the PRV infected farms were purchasing breeding animals (gilts or/and boars) from sources outside the farm and 100% of them did not apply quarantine for the newly purchased breeding animals!

Table 3. 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
Gastroenteric 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

Moreover, according to the results of the multivariable logistic regression analysis, 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 or to 30.8% when farms were located in high-density areas and were applying hygienic/biosecurity measures. 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. 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 flying insects, as well as of other wild and domestic animals should play a role in the spread of PRV in high-density areas.

MCA had shown (table 4) that the presence of wild-type PRV is mostly related with problems in growing and finishing pigs, as well as is also related to respiratory and reproductive disease problems and increased mortality. Lack of appropriate biosecurity measures and economical problems may be substantial factors for such presence.

Table 4. Multiple correspondence analysis (MCA)

CTR	F1			
37	-283	D32	Problems in growing pigs	6
37	-418	A11	Presence of PRV	7
34	-273	C42	Presence of respiratory problems	8
34	-386	D42	Problems in finishing pigs	9
29	-408	C22	Increased mortality	13
28	-283	B81	Abscence of biosecurity measures	14
23	-247	C62	Presence of reproductive problems	17
22	-196	C12	Presence of general health problems	19
22	-209	B52	Presence of economical problems	20
21	-225	A41	Presence of A. pleuropneumoniae	21

In conclusion, this study provides new information regarding the presence of PRV in Greek pig farms. The use of such information may assist in designing and implementing measures to control and eradicate the disease from Greece.

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