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Serological study of potential zoonotic pathogens in Iberian pigs

J. Gómez Laguna*, M. Hernández García*, R. García-Valverde*, P.J. Moreno Moreno, B. Huerta Lorenzo*** and R.J. Astorga Márquez*****

*Centro de Investigación y Calidad Agroalimentaria del Valle de los Pedroches, CICAP Pozoblanco, Ctra. De la Canaleja s/n, 14400 Córdoba (Spain)
**Technical Services iberian pigs, Cooperativa del Valle de los Pedroches S.C.A., COVAP, Pozoblanco, Córdoba (Spain)
***Animal Health Departmet, Veterinary Faculty, University of Córdoba, Córdoba, (Spain)

Abstract. Zoonotic diseases can course as inapparent diseases in porcine livestock, however, they present a significant impact in the field of public health. These diseases include *Brucella* and *Salmonella* infections and infestations with *Toxoplasma* and *Trichinella*, all considered as high zoonotic risk agents by the EFSA (2010). For this reason, we performed a serological screening which allows us to know indirectly the presence and distribution of these pathogens in Iberian pigs. A total of 709 sera from 79 farms in the Iberian pig, reared in different systems were collected at slaughterhouses and kept at -20ºC until analysis. Sera were analysed with different antigens by commercial ELISA kits: *Brucella* (Ingenasa), *Salmonella* (Svanova), *Toxoplasma* (IDVet) and *Trichinella* (Prionics). The results obtained in our study allow us to conclude that *Salmonella* infection and infestation by *Toxoplasma* are widely distributed in the farms tested (73.42% and 54.43% respectively), unlike other pathogens checked, as *Brucella* and *Trichinella*, which showed low serological prevalence (3.8% and seronegative, respectively).

Keywords. Iberian pigs – ELISA – Zoonotic – Seroprevalence.

Étude sérologique de pathogènes potentiellement zoonosiques chez le porc Ibérique

Résumé. *Les maladies à caractère zoonosique peuvent suivre un cours de façon inaperçue chez le bétail porcin, mais ont cependant une répercussion très importante sur le domaine de la santé publique. Il faut souligner –parmi ces processus– les infections par* Brucella *et* Salmonella *et les infestations par* Toxoplasma *et* Trichinella*. Toutes ces maladies sont considérées à haut risque zoonosique selon l'EFSA (2010). Pour cette raison, nous avons réalisé un screening sérologique qui nous permet de connaître d'une façon indirecte la présence et la diffusion de ces agents chez le porc Ibérique. Un total de 709 sérums, originaires de 79 fermes de porcs Ibériques élevés selon différents systèmes, ont été obtenus dans l'abattoir et conservés à -20ºC, jusqu'au moment de leur analyse. Les sérums ont été confrontés à différents antigènes au moyen de kits commerciaux ELISA :* Brucella *(Ingenasa),* Salmonella *(Svanova),* Toxoplasma *(IDVet) et* Trichinella *(Prionics). Les résultats obtenus dans notre étude permettent de conclure que l'infection par* Salmonella *et l'infestation par* Toxoplasma *sont toutes deux amplement répandues dans les fermes analysées (73,42% et 54,43%, respectivement) ; au contraire,* Brucella *et* Trichinella *ont peu de prévalence sérologique (3,8% et séronégatif, respectivement).*

Mots-clés. Porc Ibérique – ELISA – Zoonoses – Séroprévalence.

I – Introduction

Zoonoses are diseases which are transmissible from animals to humans and viceversa. The modernization of the agrifood industry and the growing demand of knowledge from consumers about what do they eat has increased the necessity of control measures to obtain safe and healthy products from animals. Data from the World Health Organization (WHO) confirmed that in the last 10 years about the 75% of human diseases has been related to the presence of pathogens in products from animal origin (EFSA, 2010).

Pork is the most consumed meat per capita in Europe, followed by poultry and cattle meats (Eurostat, 2008). Spain occupies the second place in both pig and pork production and is considered as the highest pork consumer between the 27 member states of Europe (Marquer, 2010). Thus, all those aspects concerning pig health and pork safety are nowadays of significant interest first in the world market, but even with higher emphasis in Spain.

Pig livestock represents a potential risk for zoonoses due to both bacterial and parasitic pathogens. These agents may infect humans by different routes, being the most significant one the oral route or consumption of contaminated meat. Zoonotic diseases usually course as inapparent diseases in porcine livestock, displaying a significant impact in the field of public health. These diseases include *Brucella* and *Salmonella* infections and infestations with *Toxoplasma* and *Trichinella*, all considered as high zoonotic risk agents by the EFSA (2010).

The status "pork free of zoonotic agents" may favor international trades giving a differential attribute and positioning in exigent markets such as those from United States and Japan. The each time more frequent and demanded exports of Iberian pig products to foreign countries are equally affected by this situation. For this reason, a serological screening was carried out to determine indirectly, by means the detection of specific antibodies, the prevalence of these pathogens in Iberian pigs reared in different systems.

II – Materials and methods

Seventy-nine Iberian pig herds from southern Spain were randomly selected and sampled during 2008 and 2009. Sample size was assessed by the software Win Episcope version 2.0 on the basis of the number of samples required for a previous unknown prevalence (95% confidence level and 8% accepted error were assumed and confidence intervals of the prevalence were calculated).

Iberian pig herds consisted of both free-range and intensive systems, being classified within three different categories depending on the rearing system and nutritional habits (Table 1):

- Montanera (acorn-fed, AF): the animals are reared in free-range systems and they are fed only by natural resources (i.e. acorn, pasture). This period goes from October to March (next year), beginning the animals with a weight of 92-115 kg, and with a minimum average weight gain of 46 kg and a minimum period of occupation of 60 days.
- Recebo (mixed-fed, MF): the animals are reared in free-range systems but they are fed by both natural resources and commercial feed. This type of nutrition is used when there are not enough natural resources to guarantee that the animals reach the optimal weight to be slaughtered being fed only by natural resources.
- Cebo (commercial feed, CF): the animals may be reared in outdoor facilities or in intensive systems and they are fed only by commercial feed.

Table 1. Distribution of the herds and animals analysed in the different systems

	AF	MF	CF	Total
Herds	63	7	9	79
Animals	564	70	75	709

AF: acorn-fed; MF: mixed-fed; CF: commercially feed

Five to ten pigs per herd were randomly sampled, and blood samples from each were collected at the slaughterhouse. Samples were collected into evacuated tubes, allowed to clot at room temperature and centrifuged, and then the serum was harvested and frozen at -80 ºC until testing

Sera samples were analyzed for the detection of specific antibodies against *Salmonella* spp., *Brucella* spp., *Trichinella* spp. and *Toxoplasma gondii* by means of commercial enzyme-linked immunosorbent assay (ELISA) kits, following manufacturer's instructions (SALMOTYPE® Pig Screen+E, Labor Diagnostik Leipzig; Ingezim Brucella Compac 2.0, Ingenasa; PrioCHECK® Trichinella Ab, Prionics; ID Screen® Toxoplasmosis Indirect, IDVet Innovative Diagnostics). The cut-off value used for discriminating between positive and negative serum samples was 40% for *Salmonella* spp. and *Brucella* spp. antibodies, 15% for *Trichinella* spp. antibodies and 50% for *Toxoplasma gondii* antibodies.

Magnetic stirrer method for pooled sample digestion was also performed on all the animals sampled following the regulation EC-2075/2005. Briefly, one hundred grams of samples at a time (one gram from diaphragm pillar per animal) were chopped in the blender, added to the digestion fluid (1% hydrochloric acid solution + 1% pepsin in tap water) and stirred on a magnetic stirrer for 30-60 minutes at 44-46 ºC. The digest was allowed to settle for 15–20 minutes and the upper two-thirds of the fluid was decanted. The remaining fluid and deposit were poured through a 355 µm mesh screen into a conical settling glass and allowed to settle for a further 15-20 minutes. The maximum possible supernatant fluid was aspirated without disturbing the sediment, and the latter was washed with warm (37ºC) tap water and allowed to settle for another 15-20 minutes. The washed sediment was transferred to a 50-ml tube, allowed to settle, and aspirated down to a final volume of 10 ml. All 10 ml were poured into a gridded Petri dish and examined for *Trichinella* larvae with a dissecting microscope (x15-40 magnification).

Since the ELISA test and the artificial digestion yielded contradictory results to detect *Trichinella* spp., an alternative second ELISA test (ID Screen® Trichinella Indirect, IDVet Innovative Diagnostics; cut-off > 50%) was used in order to confirm the results.

III – Results

Specific antibodies against *Salmonella* spp. and *Toxoplasma gondii* were widely distributed, whereas specific antibodies against *Brucella* spp. and *Trichinella* spp. were scarcely detected. No significant differences were observed between the different rearing systems examined, however, the number of mix-fed and fed herds was not enough representative to obtain an accurate estimation.

Antibodies against *Brucella* spp. were found only in 3 out of 63 AF herds (4.76% of herd prevalence), being detected in 6 out of 564 animals (1.06% of individual prevalence). *Salmonella* spp. was more spread, being detected in all the three rearing systems with a herd and individual prevalence close to or higher than 70% and 20%, respectively (Tables 2 and 3).

Toxoplasma gondii-specific antibodies were also widely detected in all the three systems (AF, MF, and CF herds), showing a total herd prevalence of 54.43% and a total individual prevalence of 27.12%. Only three animals (1 animal per herd) showed antibodies against *Trichinella* spp. (Tables 2 and 3). However, all the sampled animals displayed negative results for routine artificial digestion. The alternative second ELISA test used to confirm those contradictory results yielded also negative results.

IV – Conclusions

The results presented in this research showed a high herd and individual prevalence for both *Salmonella* spp. (73.42% and 20.87%, respectively) and *Toxoplasma gondii* (54.43% and 27.12%, respectively) antibodies, without significant differences between the different rearing systems examined. Contrary, prevalence levels for specific antibodies against *Brucella* spp. and *Trichinella* spp. were low.

Table 2. Number of positive herds and prevalence data against each zoonotic pathogen

	Number of positive animals (prevalence)					
				Trichinella		
	Brucella	***Salmonella***	***Toxoplasma***	**ELISA 1**	**ELISA 2**	**Artificial digestion**
AF (n=63)	3 (4.76)	44 (69.84)	37 (58.73)	1 (1.59)	0 (0.00)	0 (0.00)
MF (n=7)	0 (0.00)	7 (100)	2 (28.57)	0 (0.00)	0 (0.00)	0 (0.00)
CF (n=9)	0 (0.00)	7 (77.78)	4 (44.44)	2 (22.22)	0 (0.00)	0 (0.00)
Total Herds (n=79)	3 (3.80)	58 (73.42)	43 (54.43)	3 (3.80)	0 (0.00)	0 (0.00)

AF: acorn-fed; MF: mixed-fed; CF: commercially feed

Table 3. Number of positive animals and prevalence data against each zoonotic pathogen

	Number of positive animals (prevalence)					
				Trichinella		
	Brucella	***Salmonella***	***Toxoplasma***	**ELISA 1**	**ELISA 2**	**Artificial digestion**
AF (n=564)	6 (1.06)	112 (19.86)	171 (30.32)	1 (0.18)	0 (0.00)	0 (0.00)
MF (n=70)	0 (0.00)	20 (28.57)	3 (4.29)	0 (0.00)	0 (0.00)	0 (0.00)
CF (n=75)	0 (0.00)	16 (21.33)	18 (24.00)	2 (2.67)	0 (0.00)	0 (0.00)
Total Animals (n=709)	6 (0.85)	148 (20.87)	192 (27.12)	3 (0.42)	0 (0.00)	0 (0.00)

AF: acorn-fed; MF: mixed-fed; CF: commercially feed

A high seroprevalence of *Salmonella* and *Toxoplasma*, together with minimal prevalence of *Trichinella*, has been previously reported at conventional swine production systems (Gebreyes *et al*., 2008), being observed also a higher prevalence of these pathogens in porcine reared in outdoor-herds compared to those reared in indoor-herds. In our study no significant differences were observed between the different rearing systems, however, the low number of herds sampled for MF and CF herds did not allow us to validate our results. Therefore, further studies should be conducted to carry out a more accurate approach concerning the seroprevalence in different rearing systems.

Mejía *et al.* (2006) reported a 77% of *Salmonella* herd seroprevalence in finishing pig units in Northern Spain. In addition, in a study carried out in our same geographical area a slightly higher individual (27.3%; CI_{95}: 26.1-28.4) and herd seroprevalence (80.0%; CI_{95}:71.5-86.9) of *Salmonella* was observed in pigs reared in intensive systems (Pérez-Barrios *et al*., 2010). These results are in agreement with the results presented in the present study.

Seroprevalence of *Toxoplasma gondii* has been documented worldwide, showing a frequent contact with parasite within the porcine livestock (García-Bocanegra *et al*., 2010; Hill *et al*., 2010; Veronesi *et al*., 2010). In Spain, a previous study has reported a widespread, but variable, seroprevalence of *Toxoplasma gondii* ranging from 4.4% to 27.3% in domestic pigs (García-Bocanegra *et al*., 2010). All-in all-out, cleaning measures, age, control of rodents and cats and carcass disposal methods have been related to differences in *Toxoplasma gondii* seroprevalence (García-Bocanegra *et al*., 2010; Hill *et al*., 2010; Veronesi *et al*., 2010).

Interestingly, in our study ELISA test for *Trichinella* spp. showed positive results for only three animals, however, all the animals displayed negative results by the reference method of detection (magnetic stirrer method for pooled sample digestion) (EC-2075/2005). Moreover, those doubtful results yielded also negative results by the diriment alternative ELISA test.

ELISA represent a useful a rapid method to detect the presence of specific antibodies on serum, plasma or meat juice collected before or after slaughter. *Trichinella* infestation levels as low as one larva/100 g of tissue have been detected by ELISA in pigs (Gamble *et al*., 2004). This high level of sensitivity point to ELISA as a useful method for detecting ongoing transmission of *Trichinella* spp. infection at the farm or for surveillance programmes. Although ELISA kits usually have a high sensitivity, some false-negative results may be observed due to infected animals do not develop an antibody response until 3-5 weeks post infection (Gamble, 1996). In addition, ELISA may yield a low rate of false-positive results due to the specificity of ELISA for *Trichinella* infection is variable according to the type and quality of the antigen employed in each test (OIE, 2009). This results are in agreement with the results obtained in our study and may explained the differences observed between the two ELISA kits used to detect *Trichinella* spp.-specific antibodies. For this reason, serological tests are only recommended for surveillance studies, whereas direct methods should be used to individual carcass inspection (OIE, 2009).

Interestingly, all the pathogens examined in this study has been previously found in wild boars showing similar seroprevalence trends (Gauss *et al*., 2005; Montagnaro *et al*., 2010). Therefore, measures to control the contact between domestic and wild species are especially encouraged in the plans oriented to diminish the prevalence of these pathogens.

Our results showed a wide dissemination of *Salmonella* spp. and *Toxoplasma gondii* in finishing Iberian pigs, whereas *Brucella* spp. and *Trichinella* spp. were rarely or not found. Due to the potential zoonotic risk of these pathogens, which represent a risk both at farm level and at pork level, and the present consumer's concern about food safety control measures should be adopted in order to diminish their prevalence from farm-to-fork.

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References

EC-2075/2005. Official Journal of the European Union. COMMISSION REGULATION (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. L338/60-L338/82.

EFSA, 2010. The Community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. In: *EFSA Journal*, 8(1): 1496.

Eurostat, 2008. Chapter 5. Consumption stage, In: *Pocketbooks. Food: from farm to fork statistics*, p. 139-152.

Gamble H.R., 1996. Detection of trichinellosis in pigs by artificial digestion and enzyme immunoassay. In: *J. Food Prot*., 59: 295–298.

Gamble H.R., Pozio E., Bruschi F., Nöckler K., Kapel C.M.O. and Gajadhar A.A., 2004. International Commission on Trichinellosis: Recommendations on the Use of Serological Tests for the Detection of Trichinella Infection in Animals and Man. In: *Parasite*, 11: 3–13.

García-Bocanegra I., Simon-Grifé M., Dubey J.P., Casal J., Martín G.E., Cabezón O., Perea A. and Almería S., 2010. Seroprevalence and risk factors associated with *Toxoplasma gondii* in domestic pigs from Spain. In: *Parasitol. Int.* 59(3): 421-426.

Gebreyes W.A., Bahnson P.B., Funk J.A., McKean J. and Patchanee P., 2008. Seroprevalence of *Trichinella*, *Toxoplasma*, and *Salmonella* in antimicrobial-free and conventional swine production systems. In: *Foodborne Pathog Dis*. 5(2): 199-203.

Hill D.E., Haley C., Wagner B., Gamble H.R. and Dubey J.P., 2010. Seroprevalence of and risk factors for *Toxoplasma gondii* in the US swine herd using sera collected during the National Animal Health Monitoring Survey (Swine 2006). In: *Zoonoses Public Health*. 57(1): 53-59.

Marquer P., 2010. Pig farming in the EU, a changing sector. In: *Statistics in focus. Eurostat*.

Mejía W., Casal J., Zapata D., Sánchez G.J., Martín M. and Mateu E., 2006. Epidemiology of *Salmonella* infections in pig units and antimicrobial susceptibility profiles of the strains of *Salmonella* species isolated. In: *Vet Rec.* 159(9): 271-276.

Montagnaro S., Sasso S., De Martino L., Longo M., Iovane V., Ghiurmino G., Pisanelli G., Nava D., Baldi L. and Pagnini U., 2010. Prevalence of antibodies to selected viral and bacterial pathogens in wild boar (*Sus scrofa*) in Campania Region, Italy. In: *J Wildl Dis*. 46(1): 316-319.

OIE, 2009. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.1.16 Trichinellosis, p.344-352.

Pérez-Barrios F., Borge C., Astorga R.J., García Bocanegra I., Carbonero A., Arenas A. and Perea A., 2010. Seroprevalence and risk factors of Salmonella spp. in swine farms from Southern Spain. In: *I3s International Symposium on Salmonella and Salmonellosis*. Saint Malo, France. 28th-30th June: 259.

REAL DECRETO 1469/2007, de 2 de noviembre, por el que se aprueba la norma de calidad para la carne, el jamón, la paleta y la caña de lomo ibéricos.

Veronesi F., Ranucci D., Branciari R., Miraglia D., Mammoli R. and Fioretti D.P., 2010. Seroprevalence and risk factors for *Toxoplasma gondii* infection on finishing swine reared in the Umbria region, central Italy. In: *Zoonoses Public Health.* doi: 10.1111/j.1863-2378.2010.01336.x