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INFECTIOUS DISEASE STATUS IN WILD BOAR PIGLETS:

A MISSING LINK

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ABSTRACT

As wild boar populations continue to rise, both in numbers and in range worldwide, so does the role that this species plays as a carrier of important infectious agents. In the following study, we tried to conduct a mapping of lethal infectious agents in wild boar, and specifically in piglets, alias the youngest age class, in southern-central Spanish semi-intensive management conditions. The objectives of the following study were: 1) to investigate infection status of wild boar piglets in semi-intensive game estate conditions, 2) to determine links between certain pathogens and the high mortality rates in wild boar piglets in semi-intensive game estate conditions and 3) to discover co-infection interactions and relationships between certain pathogens in wild boar piglets.

To accomplish the above objectives, 392 wild boar piglets were captured and underwent blood sample collection. Also 182 carcasses of wild boar piglets which died during weaning in intensive management facilities, were collected, transferred to laboratory facilities, and underwent a full-scale necropsy examination, which included lung consolidation percentage as well as tuberculosis gross lesions scoring. Extracted samples, along with field harvested samples were furthermore tested by ELISA for *Mycobacterium tuberculosis* complex (MTC) antibodies and cultured for the same agent. In addition, selected samples were tested using serological methods, and also by PCR for detection of PCV2 and *Mycoplasma hyopneumoniae*. Finally, statistical tests were conducted to process results and to unmask pathogen links and interactions.

The piglets were found 34% and 37.5% positive for MTC, by culture and ELISA, respectively. A low mean of 1.3 (scale 0-26) was found in TB-lesions severity scoring. In addition, a relative medium to high score of PCV2 prevalence was detected (26% by PCR and 31% by ELISA), and a relatively high score of *M. hyopneumoniae* infection (18% by PCR). Moreover, necropsy revealed a high rate of lung consolidation lesions, with a mean of 17% of the lungs with EP-like lesions. Intestinal parasites were also detected with a high frequency (14%). Also concerning the GI tract we found evidence of frequent infection by intestinal pathogens (intestinal lesions in a total of 18%). Finally, indicators of high levels of chronic stress were detected (external hematomas, gastric ulcers). From the above it seems that infectious agents of the respiratory system together with intestinal pathogens and high stress levels seem to play a vital role in defining mortality rates in wild boar piglets.

Finally, through statistical analysis, complex links between agents were discovered, as PCV2 infection and parasitic load seem to induce immunosuppresion, thus paving the road for more severe chronic respiratory and intestinal infections by various agents, such as *M. hypneumoniae* and synergistic MTC invasion.

KEYWORDS: Tuberculosis; Co-infection; Porcine circovirus type 2; Eurasian wild boar; piglets; *Mycobacterium bovis*; Semi-intensive management; *Mycoplasma hyopneumoniae*; Macroparasites

INTRODUCTION

Wild boar (or feral pig in the USA; *Sus scrofa*) an ancestor of the common pig, native in Eurasia, is nowadays a species with worldwide distribution (excluding Antartica, Lang et al. 2003), and one of the most abundant mammals in the world.

All these populations have expanded throughout Europe in the last century, both in distribution as well as in abundance (Acevedo et al. 2007). This expansion can be attributed to human migration towards urban areas and consequent reductions in hunting pressure, and increased maize production as well as other factors. With the expansion of this species, and with the economic importance which it has gained, especially towards big game recreational hunting in several places, it gained weight as an important carrier of various pathogens as well.

Thus, while wild boar currently flourishes carrying diseases both of public health and of livestock industry importance, unfortunately, wildlife disease monitoring has not followed the same trend, as few European countries have comprehensive wildlife disease surveillance schemes (Kruger 1998, Acevedo et al. 2006, Tsachalidis et al. 2008, Gortazar et al. 2012).

Although there has been thorough research and monitoring of disease status in adult wild boar populations, the same cannot be stated for the total of wild boar life cycle, as available data is scarce concerning early age (less than 6 months to one year old individuals) disease status of this species. The explanation of this phenomenon is easy, as almost the total amount of studies (always with few exceptions like this one) harvest their data from hunting bags and in almost every country where hunting is practiced, a very small amount of piglets is hunted every season. The prior is either due to regulations against hunting piglets, or/and due to the general dislike of hunters to shoot female individuals with piglets, which also do not comprise appraisable trophies.

Piglet mortality

In modern swine industry it is a fact that piglet mortality is a major cause of both economic loss and poor welfare. It is calculated that mortality increases with litter size and ranges from 5-45%, with a mean of 15%, depending on various conditions (Killbride et al. 2014, Mellor et al. 2004).

In wild boar, piglet mortality in natural conditions, according to Keuling et al. (2013), reaches a mean of 50%. In the conditions of central Spanish hunting estates, with supplementary feeding and high aggregation piglet mortality can rise up to 70% (our case) according to Armenteros thesis project (2013) who performed the above analysis. With mortality rates of this magnitude it is obvious that economic loss is major, and that steps have to be made to locate causality of the situation, in order to try solving the problem and eventually lowering rates.

Disease status

Wild boar, as aforementioned can carry diseases that are important not only for domestic swine and livestock industry, but also to public health and even to wildlife conservation.

A common method to classify diseases is by the type of the pathogen-s which may cause them. So, in wild boar, pathogens, according to their type, can be grouped as viral, bacterial and parasitic.

Crucial viral pathogens identified as prevalent in wild boar consist of Hepatitis E virus (HEV), pseudorabies virus (PRV), classical swine fever virus (CSFV), African swine fever (ASFV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine reproductive and respiratory virus (PRRSV), torque teno virus (TTV) and Japanese encephalitis virus (JEV).

Furthermore, important bacterial and parasitic pathogens known to frequent wild boar include *Mycobacterium bovis, Mycobacterium avium, Leptospira interrogans, Yersinia pestis, Francisella tularensis, Mycoplasma hyopneumoniae, Escherichia coli, Pasteurella multocida, Actinobacillus pleuropneumoniae, Coxiella brunetii* and *Brucella suis* while parasites include,-Trichinella spiralis and Toxoplasma gondii- for instance (Ruiz-fons et al. 2008, Meng et al. 2009).

While the above pathogens frequent adult wild boar, the image becomes a lot grayer when it comes to juvenile and piglets, as most prevalence studies do not provide figures for this age. So, a crucial objective of the current study was to decode disease status in piglets and to link mortality rates with certain pathogens and disease complexes (as, to construct a realistic portrait of today's infection status in livestock and wildlife together using isolated pathogens would be over-simplistic).

It is a fact that in livestock health studies nowadays pathogens are not treated in the context of units, but preponderantly in the context of groups-complexes as it has been revealed that most times they act as a sum, having synergistic effects, in order to produce substantial results, e.g. clinical signs and lesions.

On the contrary, this concept is a lot more vestal in wildlife disease monitoring, but is also being established as a predominant concept in this field. Considering the above, there are situations where even more complex pathogen relationships can be created when wildlife and livestock intermingle in variable environmental conditions, making it a challenge to unwind.

In domestic swine, the pathogens that contribute to neonatal and piglet mortality mostly belong to either respiratory disease complexes or enteritis disease complexes, with the symptoms that defy every complex (yet, some symptoms may be identical in a

variety of infections, like lameness, low weight gain, etc.). Based on domestic piglet mortality, table 1 was compiled, where a list of possible contributing pathogens is being cited.

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Table 1. Essential pathogens that may contribute to wild boar piglet mortality rates

RESPIRATORY DISEASES COMPLEX	ENTERITIS DISEASES COMPLEX	OTHER DISEASES
ACTINOBACILLUS PLEUROPNEUMONIAE	*ESCHERICHIA COLI*	*ERISYPELOTHRIX RHUSIOPATHIAE*
MYCOPLASMA HYOPNEUMONIAE	*SALMONELLA CHOLERASUIS*	PSEUDORABIES VIRUS (PRV)
PORCINE REPRODUCTIVE RESPIRATORY SYNDROME (PRRSV)	CLASSIC SWINE FEVER (CSFV)	PORCINE PARVOVIRUS (PPV)
PASTEURELLA MULTOCIDA	TRANSMISSIBLE GASTRENTERITIS VIRUS (TGEV)	
SWINE INFLUENZA		
MYCOBACTERIUM TUBERCULOSIS COMPLEX (MTC)		
PORCINE CIRCOVIRUS TYPE 2 (PCV2)		

From table 1, but also in general, Porcine circovirus type 2 virus (PCV2) and *Mycoplasma hyopneumoniae* bacteria are agents found frequently to contribute in domestic piglet mortality. But albeit domestic, they have also been detected as disease contributors in wild boar.

Mycoplasma hyopneumoniae

Domestic swine

Mycoplasma hyopneumoniae is the etiologic agent of EP (enzootic pneumonia) in domestic swine. EP tends to be a disease of high morbidity and low to medium mortality, and with high economic impact upon swine production. It is considered by many as the most economically significant porcine bacterial respiratory pathogen. In domestic swine the organism is primarily found on the mucosal surface of the trachea, bronchi, and bronchioles. Main clinical sign is non-productive coughing, and macroscopic lesions, consisting of purple to grey areas of pulmonary consolidation, are mainly found bilaterally in the apical, cardiac, intermediate and the anterior parts of the diaphragmatic lobes (Maes et al. 2008).This disease affects swine, from growers to finishing pigs, mainly from 8 to 26 weeks of age, while the colonization, presumably, occurs during weaning (Fano et al. 2007).

Wild Boar

In wild boar the pathogen has been found in sera and tissue throughout Europe (table 2). From the above reports, three state that they have found antibodies in young individuals. Closa-Sebastia et al. at 2011 found a seroprevalence for M.hyo of 8.5% in piglets (individuals from 0-6 months) with a ratio of 4/47, and Sibila et al. 2009 in weaning wild boar piglets found 18% ELISA prevalence 17/95 for M.hyo. In the case of Sibila et al. 2009, M.hyo DNA was also detected by nPCR in 5 out of 17 (29.4%) nasal swabs and in 5 out of 29 (17.2%) lung samples. Also, presence of EP-like microscopic lung lesions was observed in 3 out of 10 (30%) animals, but no gross macroscopic lesions in lungs were observed.

Table 2. *Mycoplasma hyopneumoniae* prevalence studies in European wild boar

PREVALENCE	***COUNTRY***	***METHOD/RATIO***	***AUTHORS***
58%	France	Serology 53/91	Marois et al. 2006
52%	Russia	Serology 51/98	Kukushkin et al 2009
30%	Italy	Serology 665/2177	Chiari et al. 2014
26.6%	Catalonia – NE Spain	Serology 71/267	Closa-Sebastia et al. 2011
21%	Slovenia	Serology 38/178	Vengust et al. 2006
15.84%	Slovenia	Serology 29/184	Stukelj et al. 2014
8% **20%**	Spain	PCR 12/156 lung 17/85 nasal	Sibila et al. 2009
21%	Spain	Serology 92/428	Sibila et al. 2009
13.94%	Spain	Serology 23/165	Risco et al. 2014

Yet, with the studies of Ecco et al. (2009) in farmed wild boar in Brazil, and more recently Chiari et al. (2014) in free-roaming wild boar in Italy, it has been proved that *Mycoplasma hyopneumoniae* does produce macroscopic lesions and thus disease in this species, as it was confirmed not only by interstitial bronchopneumonia images in necropsies, but also with PCR from lungs with severe lesions. Furthermore Chiari et al., (2014) found that younger individuals had higher prevalence, and that infection occurs in early age and generally becomes chronic.

To conclude, *Mycoplasma hyopneumoniae* seems to establish early infection and to be of significant importance for wild boar piglets. The detection of its DNA and presence of both micro- and macroscopic lesions compatible with EP suggest that, besides infection, the pathogen is capable of causing broncho-interstitial pneumonia and clinical signs and disease in wild boar, adults and piglets together. Finally, taking into account the molecular studies of Kuhnert et al. (2014), who discovered identical genotypes of Mycoplasma strains from EP outbreaks in Switzerland between wild boar and domestic pig, its potential role as a reservoir for the domestic pig cannot be ruled out, and is somewhat strengthened, but yet remains to be explored furthermore.

Porcine circovirus type 2 (PCV2)

Domestic swine

Porcine circoviruses (PCV) are present in pigs worldwide and have been associated with a number of syndromes such as postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex, and reproductive disorders. The term “porcine circovirus diseases” (PCVD) was proposed to group diseases or conditions linked to PCV2. Among PCVD, only PMWS has a worldwide impact on swine production. PMWS is considered the most significant PCVD, and most of the prevention/control and research efforts have been focused on it.

Oronasal exposure is considered the primary route of transmission, but PCV2 has been found in nasal, tonsillar, bronchial, and ocular secretions, feces, saliva, urine, colostrum, milk, and semen. Longitudinal studies quantifying PCV2 in serum, nasal excretions, and feces found that most piglets became infected at 4–11 weeks of age, depending on the farm. Infectious agents considered potential PMWS triggers include infection with porcine parvovirus (PPV) and/or porcine reproductive and respiratory syndrome virus (PRRSV), and non-infectious include vaccinations, nutrition and management practices, and stressful conditions in general. In the field most PCV2 infections are subclinical. PMWS most commonly affects pigs at 2–4 months of age. PCV2-infected pigs and their viral load increase gradually after lactation and coincident with the decline in maternal immunity. Almost all the pigs in a PMWS-infected farm will become infected with PCV2 but only a small portion will develop PMWS. The above condition is clinically characterized by wasting, respiratory distress, pallor of the skin and, less frequently, jaundice (Segales et al. 2005).

Wild Boar

From table 3 prevalence rates, it is conclusive that PCV2 seems to be present in high rates in wild boar populations, and also seems to be widespread and endemic in the wild boar populations in various countries (Vicente et al. 2004. Morandi et al. 2010). Nevertheless, the infection rates of wild boar appear to be clearly lower than those of domestic swine, which in many cases near 100%. This is speculated to happen due to the different environments that the two species live in. Factors such as living conditions, age of infection, extent of PCV2 shedding, early weaning, and vaccinations (Vicente et al. 2004), factors that may enhance the spread of the virus in domestic swine, may not be applicable to wild boar populations. It has been proved that in wild boar, both seroprevalence and level of antibody titers are density-dependent, and thus higher in intensive management or/and fenced environments (Vicente et al. 2004. Ruiz-Fons et al. 2006). It has also been shown that wild boar/feral pig seroprevalence is proportional to the density of domestic swine in an area (Corn et al. 2009, Reiner et al. 2010).

Table 3. PCV2 prevalence studies in European wild boar

PREVALENCE	***COUNTRY***	***METHOD/RATIO***	***AUTHORS***
31.6%	Belgium	Serology 100/281	Sanchez et al. 2001
47.87%	Spain	Serology 314/656	Vicente et al. 2004
51.8%	Spain	Serology 141/272	Ruiz-Fons et al. 2006
43%	Czech Republic	Serology 57/134	Sedlak et al. 2008
39.8%	Italy		Delogu et al. 2008
10.9%	Italy	Immunofluorescence-Immunohistochemistry 38/348	Morandi et al. 2010
5.3%	Spain	In situ hybridization 3/56	Vicente et al., 2004
18.1%	Germany	PCR 43/238	Knell et al., 2005
63.1%	Germany	Nested PCR n=349	Reiner et al., 2010
45.4%	Germany	quantitative PCR n=349	Reiner et al., 2010
20.5%	Hungary	PCR n=2000	Csagola et al., 2006
25%	Slovenia	PCR spleen	Toplak et al. 2004
43.86%	Romania	PCR 93/212	Turcitu et al. 2011

Concerning porcine circovirus diseases complex, the fact has been established that wild boar can be affected by PMWS. There have been multiple reports of individuals, or small groups of these animals who have met the three criteria, thus verifying infection by PMWS, and the presence of the syndrome in this species (Ellis et al. 2003, Vicente et al. 2004, Correa et al. 2006, Lipej et al. 2006, Sofia et al. 2008, Petrini et al. 2009, Morandi et al. 2010, Reiner et al. 2010 Borba et al. 2011).

Whereas PMWS mainly affects piglets 2-4 months of age, wild boar piglets [although there have been cases of wild boar piglets 2-4 months old proven to be infected by PMWS (Sofia et al. 2008)] mainly become affected by the sydrome after the 6^{th} and between the $7\text{-}8^{th}$ month of age (Schultz et al. 2004, Vicente et al. 2004, Morandi et al. 2010). This delay may happen due to the later weaning age of wild boar in nature (Correa et al. 2006) or to the absence of intensive management procedures such as vaccinations and other stressors (Morandi et al. 2010).

The clinical signs of PMWS in wild boar appear to be identical to those of domestic swine, in particular poor body condition with variable depletion of fat stores and muscular atrophy, respiratory distress, ocular discharge, diarrhea, pallor of the skin and evidence of macroscopical wasting. Gross lesions that have been described include mildly to moderately enlarged lymph nodes and spleen, petechial hemorrhages on the kidney cortex, interstitial pericarditis, ulceration of the fundic portion of the stomach, edema of the small bowel equivocal linear erosions in the ileum and spiral colon, ascites, hydrothorax, and non collapsed lungs, cranioventral consolidation and interlobular edema of the lungs, interstitial pneumonia with congestion and edema. Main histological findings include lymphocyte depletion with moderate histiocytic infiltration of lymphoid tissues, hyperplasia of lymphoid aggregates and dilatation of lymphatic vessels in the intestines, macrophage infiltration, multinucleate giant cells organised into micro-granulomas in follicle centres and/or peritrabecular space, and interstitial pneumonia often complicated by necrosis and fibrinous exudate (Ellis et al. 2003, Vicente et al. 2004, Correa et al. 2006, Lipej et al. 2006, Sofia et al. 2008, Petrini et al. 2009, Morandi et al. 2010, Reiner et al. 2010, Borba et al. 2011).

Whether PCV2 can also cause reproductive disorders in wild boar as it does in domestic swine remains unanswered. Although PCV2 has been traced in the uterus of a pregnant female wild boar which was proven to be infected with PMWS, viral DNA or any type of abnormalities were not found in the two fetuses which the animal carried, thus deeming the results inconclusive (Ruiz-Fons et al. 2006, Sofia et al. 2008).

Along with PMWS infection, individuals or groups of animals have been found co-infected with various pathogens, such as *Mycoplasma spp*., *Streptococcus suis* (Ellis et al. 2003), PRRS (Sofia et al. 2008), *Salmonella cholerasuis, Pasteurella multocida, Metastrongulus apris, Ascaris suum, Trichuris suis* (Lipej et al. 2006), *Pneumocystis spp., Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans* (Zlotowski et al. 2011), further supporting the theory that PCV infection induces immunosuppresion which favors secondary co-infections from various pathogens (Petrini et al. 2009) , a

theory that is strongly suspected for domestic swine (Segales et al. 2004). Moreover, it is being speculated that stress due to management issues, even if not the sole factor, is a factor which plays a role in the severity and pathogenicity of PMWS infection in farmed and/or fenced animals (Ellis et al. 2003, Vicente et al. 2004).

Although there have been recorded mortality rates of 20-50% due to PMWS (Lipej et al. 2006, Correa et al. 2006, Castro et al. 2012) among wild boar piglets in fenced estates and in farmed animals, the precise epidemiologic effect that PCV-diseases induce upon wild boar and specifically the contribution of PMWS in wild boar piglet wasting and mortality rates remains unclear. Yet, it is speculated that PCV does not affect wild boar as much as domestic swine. The above is supported not only by the lower seroprevalence of PCV in wild boar, as stated above, but also by the fact that the level of PCV viral load in wild boar overall appears to be lower than in domestic swine, lower than the threshold of disease occurrence (10^7 copies per 500 ng of sample DNA), thus maintaining a subclinical infection in the majority of pcv-infected wild boar population (Reiner et al., 2010). However, if PMWS proves indeed of importance in wild boar piglets, the control of the disease would be very difficult in wild boar populations since the control of the disease in domestic swine is being carried out through all in-all out management, strict hygiene and other husbandry measures, which cannot be applied in wild boar (Vicente et al. 2004).

Addressing the question of reservoir status of wild boar for PCV-2, it has been proven that transmission of viral PCV2 DNA from wild boar to domestic swine does occur (Reiner et al. 2011, Cadar et al. 2012). Yet the higher diversity of wild boar PCV2 genotypes, (based on which Cadar et al. at their study in 2012 address the possibility of recombination between PCV2 strains of wild boar and domestic pig origin, as they prove the existence of ORF2-based intra-and inter-genotype recombination within wild boar populations), the rarity of more than 50% of wild boar PCV2 genotypes among domestic swine worldwide, along with the near complete absence of PCV2a-c strains in domestic swine (strains which are still frequent in wild boar population with a prevalence of more than 50%), further strengthen the hypothesis that the level of exchange is low, and that virus infections of swine have a higher probability to spread from domestic pigs to wild boar rather than the reverse (Albina et al. 2000, Reiner et al. 2011, Cadar et al. 2012). The above facts along with the difference in sero-prevalence of PCV2, (also mentioned above) between the two species, lead to the assumption that domestic pigs are probably the reservoir for PCV2 infection and may spread the disease to wild boars and to a lesser extent, vice versa. (Correa et al., 2006 Lipej et al., 2006 Morandi et al. 2010, Reiner et al. 2011, Cadar et al. 2012). Yet further studies, predominantly of PCV2 seroprevalence and genotype, are necessary in order to reach for more concrete evidence.

Mycobacterium tuberculosis complex (MTC)

Domestic swine

Swine are susceptible among other species of Mycobacteria, to *Mycobacterium avium complex* and to MTC. Although MTC has been nearly eradicated in many developed European countries, through cattle control and slaughter programs, in the production line lesions from lymph nodes continues to appear thus causing significant economic loss. Nevertheless, the increasing spread of outdoor pig farming systems has led to the re-emergence of *M. bovis* infection (Bailey et al. 2013). While tuberculosis is present in swine, no tuberculin (or other type of) test confirmed monitoring data exist, albeit data from macroscopic inspection during meat processing. For *Mycobacterium bovis*, domestic cattle are considered as main source of infection for domestic swine (Thoen. 2012). Generally, no clinical signs appear, mostly only foci of tuberculous lesions in various lymph nodes and at the intestinal tract, due of the low infection status. As tuberculosis is a disease with a long chronic route, gross lesions in swine increase by age (Pesciaroli et al. 2014).

Wild boar

Tuberculosis in wild boar nowadays seems to qualify as a research hot-spot, mainly due to three reasons. Due to the importance of the disease for public health and for cattle industry both, to the increasing population trends and worldwide spread of wild boar and feral pig, and finally due to the fact that the role of wild boar as a reservoir for TB in numerous locations has been identified and widely accepted (Hardstaff et al. 2013, Palmer. 2013).

In central and south of Spain in big game hunting estates, it seems that *M.bovis* circulates with a very high prevalence (with aggregation being a very crucial factor of the aforementioned situation), as high as 63% in a recent study by Vicente et al (2013).

The primary route of infection seems to be oral (Martin-Hernando et al. 2007), and animals can get infected from very young age, as lesions have been discovered in all age groups. But, in agreement with domestic swine, an increasing trend exists towards age groups, with adult boar having the highest prevalence, (Gortazar et al. 2008, Vicente et al. 2013). Generalized disease (i.e. lesions in > 1 anatomic region) can be seen in > 50% of tuberculous boar.

Lesions in wild boar piglets seem to be identical to adult individuals, but with lower severity and spread, and include tuberculous foci and granulomas of variable size in lymph nodes, with head lymph nodes and in particular the mandibular ones having the highest prevalence. Foci are also less frequently found in tonsils, lungs, spleen, and liver (Martin-Hernando et al. 2007).

Disease co-infection links

As in natural conditions individuals get in contact with various pathogens which may act with synergy or antagonism, nowadays we witness a shift from one host-one pathogen concept towards more complex multi-synergic approaches, in an effort of understanding further and thus solving situations of infection and disease.

Following this route, *Mycobacterium tuberculosis* complex was recently found to co-interact with PCV2, *Metastrongylus spp*., and PRV (Risco et al. 2014, Diez-Delgado et al. 2014). Specifically, individuals co-infected with these pathogens seem to suffer more severe tuberculosis infection, both in terms of lesion extent and prevalence. Moreover, in both studies, correlation between age and severity of PCV2-*M.bovis* co-infection was found, not only showing that the above situation affects young individuals most, but furthermore allowing the authors to speculate that early PCV2 infection could impair the ability of wild boar piglets to respond to other infections, including MTC (Diez-Delgado et al. 2014).

Considering the above, the value of a study concerning wild boar piglet disease status and biological, biometric interactions in variable environmental situations, would be high not only in terms of unmasking disease status and interaction with livestock, but also in terms of understanding co-infection links and interaction between pathogens, and furthermore to reduce wild boar piglet mortality in semi-natural and farmed game estate conditions.

Therefore, the objectives of the current study are:

1. To investigate infection status of wild boar piglets in semi-intensive game estate conditions.

2. To determine links between certain pathogens and mortality rates in wild boar piglets in semi-intensive game estate conditions.

3. To discover co-infection interactions and relationships between certain pathogens in wild boar piglets.

MATERIALS AND METHODS

STUDY AREA

Our study was conducted in 4 different farms in Sierra Norte de Sevilla, Andalucia region in Southwestern Spain (37º56'01" N, 5º31'32" W). The area of study was a total of 6000 ha, and all the farms were fenced. The whole study area is dedicated to big game hunting, and utilizes a semi-intensive management system. The management system includes supplementary feeding and early weaning of the piglets. Supplementary feeding occurs during the period of greatest shortage (approximately May to November), consists primarily of corn, and is delivered daily through feeders throughout the whole of the area.

WEANING MANAGEMENT

For the first time (to the best knowledge of the author) a stage-management system similar to porcine industry standards was applied and tested at wild boar, in order to reduce the high mortality rates observed in early age. Wild boar piglets were captured, maintained in captivity for 4-5 months, and then freed.

Figure 1: Selective trap-feeder with modified doors allowing only animals of certain size to enter.

In particular, the piglets were captured using weight-activated traps, specially modified in order to permit entrance only to small sized animals *(Figure 1).* Capturing took place during June-July of two subsequent years, using supplementary feeding as bait

which was placed inside the traps. Afterwards, the animals were placed in installations similar to those used for weaning/transition of domestic piglets, utilizing organization in cells (*Figure 2*). The animals remained in this facility for 4-5 months during which they were fed 3 types of forage according to their age (calculated in approximation): prestarter (during the first 40 days of age), starter (80 days of age) and finally, gain (until their release). Ultimately, the animals were periodically released during November, depending on weather conditions.

SAMPLING AND DATA COLLECTION

Blood samples (n=392) were harvested twice from every individual captured, once during its capture and once before its release. Due to aforementioned high mortality rates among piglets, there was an abundance of carcasses retrieved from the intensive management site and also from the premises of the farms which were transferred to IREC facilities (Ciudad Real, Central Spain) and later processed.

GROSS EXAMINATION

The carcasses aforementioned underwent a thorough necropsy examination (n=182) utilizing a detailed protocol, which included whole blood extraction, fecal sample extraction, exterior as well as interior organ inspection, collection of biometric data, tissue collection and in particular mandibular lymph nodes (LNs), tonsils, tracheobronchial LN, mediastinal LN, lungs, spleen, mesenteric LN, as well any other affected organ sample collection, inspection and storage at -20^{o} C for further processing.

Apropos the digestive tract, the protocol followed included dissection and inspection of the total tract length, as well as inspection and scoring for the presence and absence of content, using a percentage scale, evaluation of the tract content, inspection for the presence of gastric ulcers [stress and starvation indicator (Swaby et al. 2012, Amory et al. 2006)].

Referring to the respiratory system, inspection was performed starting from the upper respiratory tract, lung inspection was performed and lungs were furthermore weighed, palpated and scored by lobe for pneumonia-type lesions using a weighted percentage scoring system (Sibila et al. 2014, Detmer et al. 2013, Halbur et al. 1995). Also samples from every lung lobe were extracted and stored for further testing.

In relation to tuberculosis, gross examination and detection of macroscopic tuberculosis compatible lesions (any granulomatous, caseous, purulent, necrotic, calcified or proliferative types of lesions) has been evaluated as an effective method for MTC infection in this species (Santos et al. 2010, Vicente et al. 2010). Using this info, a

protocol was created that included the inspection and scoring of all relevant organs. Lesions were individually scored as 0 (invisible lesions), 1 (lesion diameter < 10 mm), or 2 (at least one lesion > 10 mm), and afterwards a total lesion score was calculated ranging from 0 to 26 points.

Lastly, external injuries, hematomas, fractures, petechiae and ecchymoses were recorded in order to further investigate their relation to social stress and food deprivation, leading to immunodeficiency and susceptibility to infectious diseases.

SEROLOGICAL STUDIES

Mycobacterium bovis and *Mycobacterium tuberculosis* complex [MTC]

The test which was used to identify piglets infected with Mycobacteria was a recently developed enzyme-linked immunosorbent assay (ELISA) which permits us to analyze large quantities of samples relatively inexpensively and thus broadens our perspective towards tuberculosis monitoring in wild boar (Boadella et al. 2011). This test uses a bovine tuberculin purified protein derivative (bPPD) as coating antigen and protein G horseradish peroxidase as conjugate and has a medium sensitivity of 79.2% and excelent specificity (100%) in detecting MTC infected wild boar. Sera from 381 wild boar piglets were tested by ELISA following the protocol described previously (Boadella et al. 2011).

PCV2

Porcine circovirus serological status was assessed by means of a commercial indirect ELISA (INGEZIM CIRCO IgG, INGENASA, Madrid, Spain). Following manufacturer instructions, the positive cut-off was calculated as the mean OD of negative controls +0.25.

Mycoplasma hyopneumoniae

Antibodies against Mycoplasma were identified also assessed by means of a commercial blocking ELISA (INGEZIM HYO COMPAC, INGENASA, Madrid, Spain). The test was conducted following protocol instructions and the cut-off values set were those proposed by the kit for differentiation (positive threshold = sample optical density (OD) 0.406 negative control.

PCR STUDIES

Extracted DNA (DNeasy extraction kit, Qiagen GmbH, Germany) from lung tissue samples, mainly from the apical lobe, was processed by standard PCV2 PCR (Quintana et al. 2002), and moreover by *Mycoplasm*a PCR. Due to logistic restrictions a sample

size of 56 animals (the total sample was selected from the animals which died during the two years of our study and were retrieved and dissected) was randomly selected and tested. The same animals were tested by PCR for both pathogens.

CULTURE STUDIES

Random selected (due to budget restrictions) samples (mandibular, thoracic lynph nodes, plus tonsils) from 53 animals were dispatched and cultured following method descriped at Garrido et al. (2011). Every grown culture with microorganisms later identified as belonging to MTC was scored as culture-positive.

STATISTICAL METHODS

For statistical correlations between continuous variables we used Spearman's rank correlation. In order to explore relationships between nominal and continuous variables Mann-Whitney U test was used. Last, for nominal variable intra-relationship exploration Pearson chi square contingency tables and Fisher's exact test were utilized. Data were analyzed using IBM SPSS statistical package version 20 (IBM Corporation, Somar, NY, USA). Kappa test by Graphpad software was used to calculate agreement between ELISA and PCR tests available at: http://graphpad.com/quickcalcs/kappa1.cfm

Figure 2: Wild boar piglet management facilities

RESULTS

During our two years of study, 392 wild boar piglets were captured, 237 in 2012 and 155 in 2013.

Tuberculosis results

In total, from the 381 wild boar piglets tested, 37.5% (n=143) were positive for antibodies against MTC by ELISA. In particular, percentages were estimated at 43.47% for 2012 (100/230) and at 28.47% for 2013 (43/151).

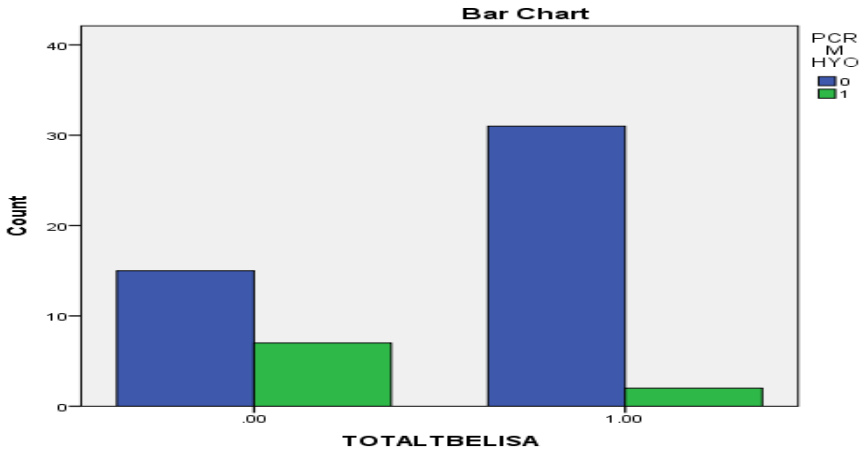
Also from the culture results, (18/53) 33.96% were cultured as *M.bovis* positive, and one sample was found positive for general *Mycobacteria* species.The TB culture results agreed with the ELISA results, tested both by chi square (chi-square=0.027) and kappa test (Kappa=0.013, SE of kappa = 0.025, 95% CI:-0.036 to 0.062).

Among the diseased animals which were necropsied, 18.68% (34/182) were classified as ‘’tuberculosis compatible’’ by macroscopic gross examination of TB lesions, and 41.2% percent (75/182) was positive for antibodies against TB bacteria by ELISA.

In addition, association was discovered between TB culture results and biometric results - thoracic perimeter (U Statistic=146.500 p=0.012) and kidney weight (with fat U Statistic=150.500 p=0.011 and without fat U-Stat=163.000 p=0.016).

Moreover, we found a statistical relationship between tuberculosis ELISA results and the PCR results for *M.hyopnemoniae*, through a pearson chi square test with a significance level set at 0.05 and for df=1 (chi-square statistic=5.285, p=0.022), which rejected our null hypothesis (graph 1).So the animals which suffered *a M.hyopneumoniae* infection tended to get co-infected more with TB than those which did not.

By the same token, the mean TB lesion score (0-26) was calculated at 1.23. Correlation was found between the level of tuberculosis lesions and the number of external hematomas (p=0.039), and also between TB and the percentage of accessory lung lobe consolidation index (p=0.041) with a strength (correlation coefficient) of 0.601 and 0.293 respectively (graph 2).



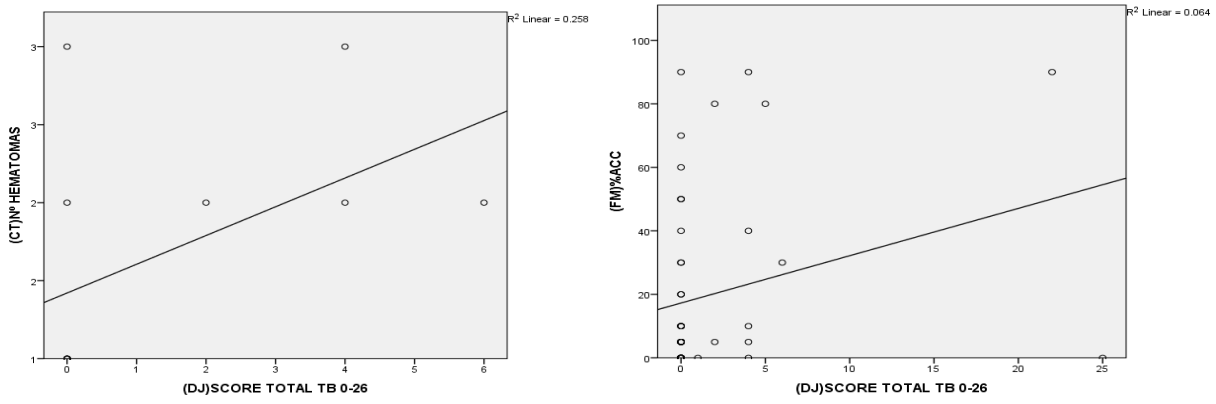
Graph 1: TB ELISA results x PCR for *M.hyopneumoniae*.

PCV2 results

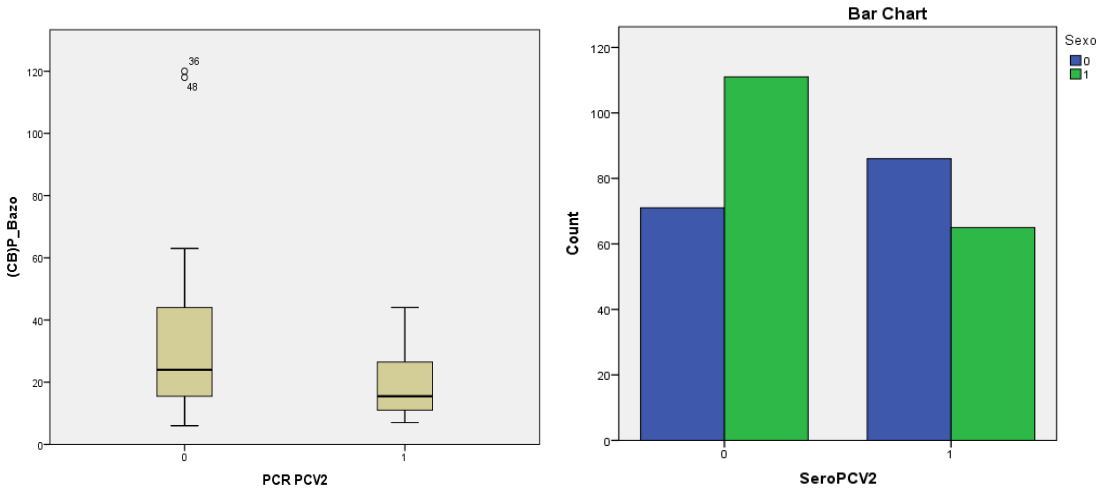
From our sample of 130 animals tested by ELISA, 25.98% (n=33) percent was found positive for antibodies against circovirus. PCR results do not seem to agree with the ELISA results (tested by Kappa=-0.039 SE of kappa = 0.052 95% confidence interval: From -0.140 to 0.062) which showed a prevalence of 30.99% (17/55).

Statistical difference of spleen weight was found between infected and uninfected groups of piglets, counted by PCR. The uninfected group was found to have a higher mean spleen weight (U Statistic=205.500, p=0.038) (Graph 3).

We also found statistical relationship between sex of the animals and serological PCV2 results, with males being more susceptible to PCV2 as the two variables did not score as independent when they were tested by chi-square (chi-square Statistic=7.987, p=0.011) (Graph 4).



Graph 2: Tuberculosis lesions score correlation graphs.

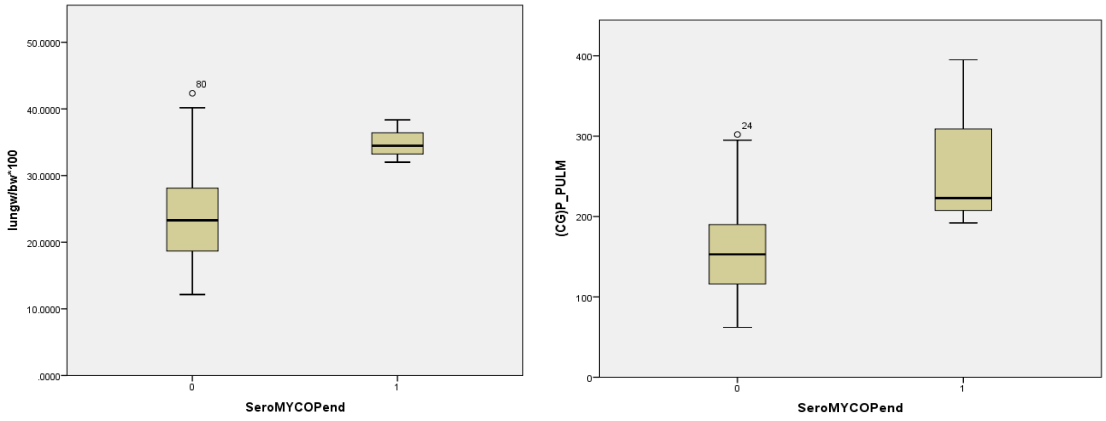


Graphs 3,4: 3. Spleen weight x PCV2 PCR. 4. Sex x PCV2 serology. M = 0, F = 1

M.hyopneumoniae results

The percentage of ELISA detected positive animals to antibodies for *M.hyopneumoniae* was 5.71% (6/105). The PCR prevalence was 18.18% (10/55). The two test results do not seem to agree (tested by Kappa=-0.090, SE of kappa = 0.042 95% confidence interval: From -0.173 to -0.007).

Regarding statistical analysis, significant difference was detected, grouping by ELISA *Mycoplasma* results, in the means of lung weight (U Statistic=20.000, p=0.027), and of one growth and development ratio (lung weight/body weight * 100)(U-Statistic=18.000, p=0.024). Males were once more detected more susceptible to *M.hyopneumoniae* by ELISA (chi-square Statistic=7.031, p=0.008), in agreement with the PCV2 serological results (Graphs 5, 6, 11).



Graphs 5,6: Mycoplasma ELISA x Growth ratio and Lung weight

The above results were also detected in PCR results, difference in the means of lung weight (U-Statistic=94.000, p=0.016) and one growth and development ratio (U-Statistic=58.000, p=0.004) (lung weight/body weight * 100). The results are plotted in graph 5. Furthermore difference was also found in the means of left caudal lobe consolidation percentage index (U-Statistic=83.500, p=0.005) (Graphs 7,8,9).

Moreover, as aforementioned, statistical relationship was found between tuberculosis ELISA results and PCR *Mycoplasma* results (Graph 1).

Last, in Fisher's exact test we detected statistical difference between infected and uninfected animals measured by ELISA antibodies, depending on cecum contents (Chi-square Statistic=5.442, Fisher Exact p=0.0.45) (Graph 10).

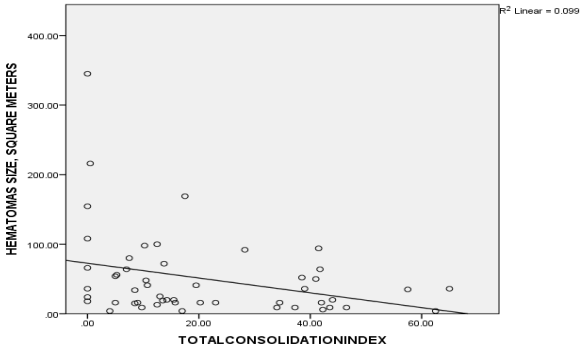
Lung consolidation

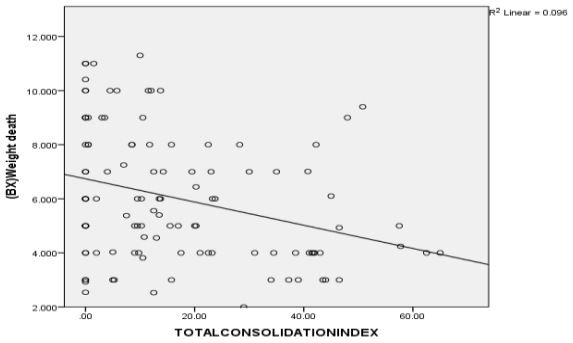
Concerning lung consolidation percentage, the total consolidation percentage was calculated from the dissection of 132 piglets, with a mean rank of 16.96%. The statistics of every lobe are shown in table 1.

Total lung consolidation percentage was found to correlate with total hematomas surface (negative strength -0.339, p=0.015, Graph 11), with the animal weight measured at death (p=0.03) with negative strength of -0.283, and also with two growth ratios, with weight/total body length ratio (negative strength -0.274, p=0.004), and with lung weight/body weight*100 ratio (strength 0.384, p=0.003)(Graphs 12,13,14). There was also correlation with other biometric data, in specific with thoracic perimeter (negative strength -0.226, p=0.017), with kidney weight (both with and without fat, with p=0.013 and 0.01, and negative strength -0.236 and -0.245 respectively, Graphs 15, 16).

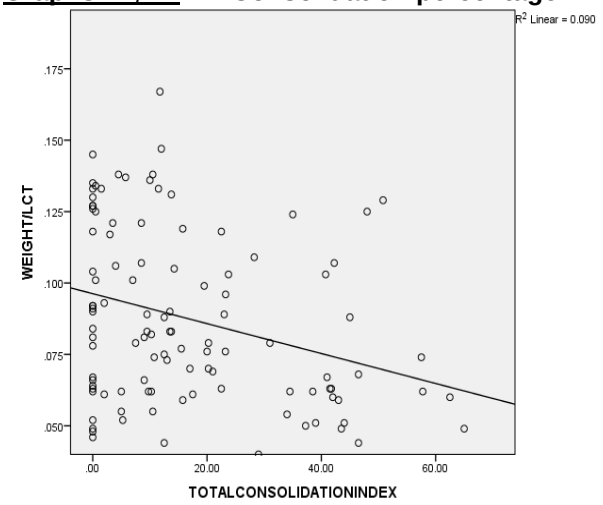
Table 1: Consolidation percentage per lung lobe

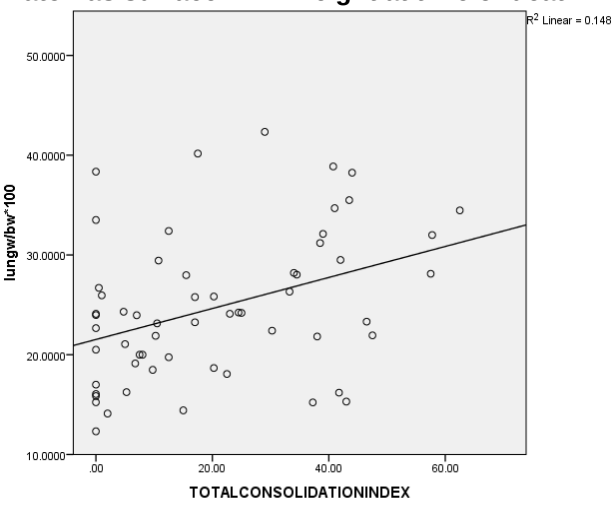
	N	Minimum	Maximum	Mean	Std. Deviation
CRANIAL LEFT-INDEX10%	132	.0000	10.0000	2.751515	3.3910408
MIDDLE LEFT-INDEX10%	132	.0000	10.0000	2.618182	3.2202086
CAUDAL LEFT-INDEX*25%	132	.00	25.00	1.6951	3.06627
ACCESORY-INDEX10%	132	.0	10.0	2.133	3.1658
CRANIAL RIGHT INDEX10%	132	.0000	10.0000	3.198485	3.5665320
MIDDLE RIGHT-INDEX10%	132	.0000	10.0000	2.893939	3.2539995
CAUDAL RIGHT-INDEX25%	132	.00	12.50	1.6705	2.79812
TOTALCONSOLIDATION INDEX	132	.00	65.00	16.9602	16.90795
Valid N (listwise)	132				

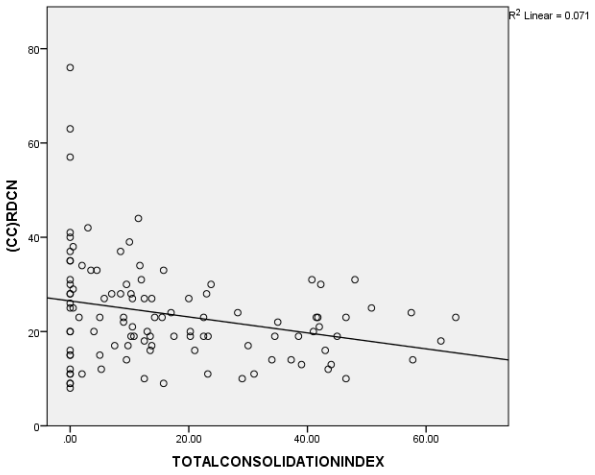


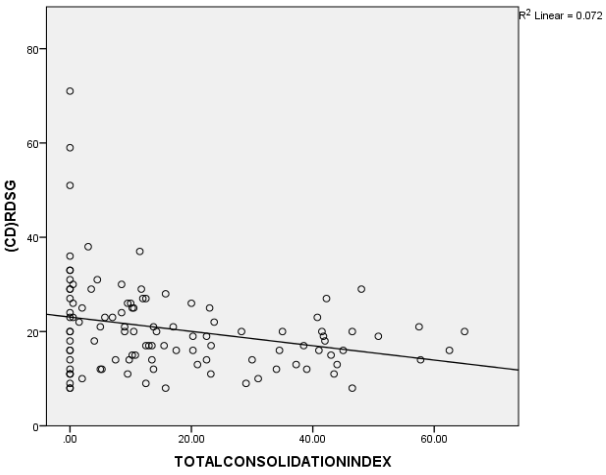


<u>Graphs 11,12:</u> 11. Consolidation percentage x hematomas surface 12. x weight at time of death

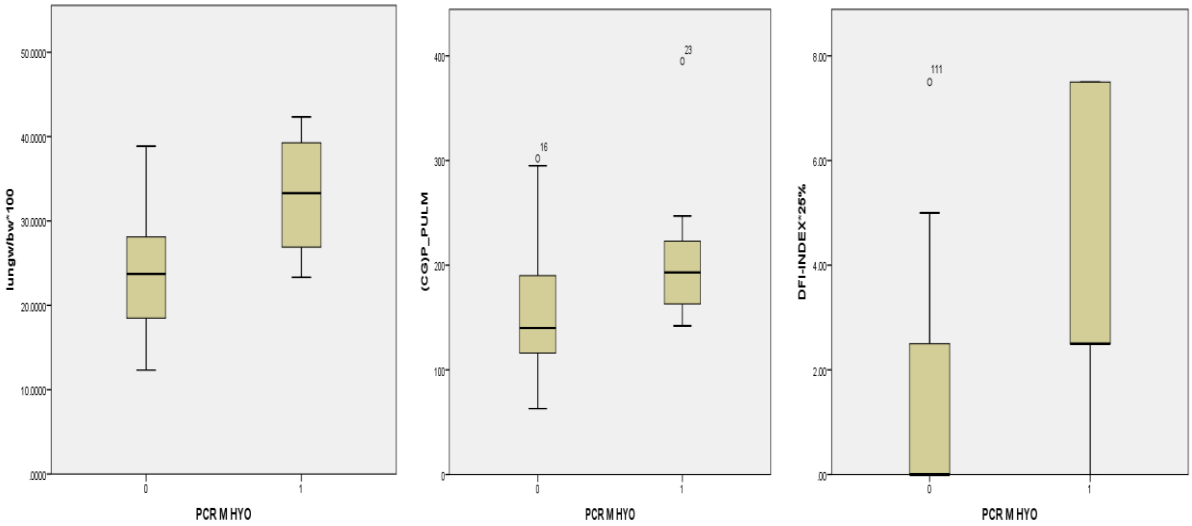




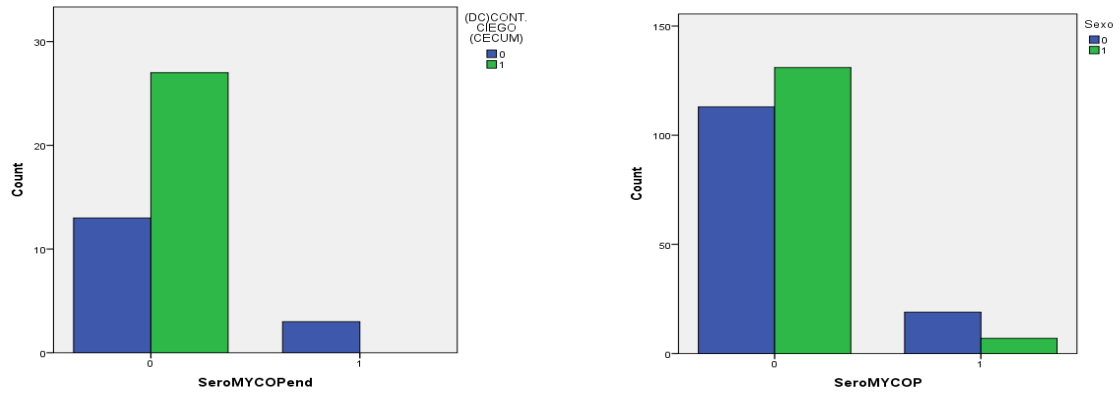




<u>Graphs 13-16:</u> Consolidation percentage x Weight/total body length 14. x Lung weigth/body weight*100 15. x Kidney weight with fat 16. x Kidney weight without fat



Graphs 17,18,19: PCR *Mycoplasma* x Growth ratio, Lung Weight, Left Caudal lung lobe consolidation score ratio



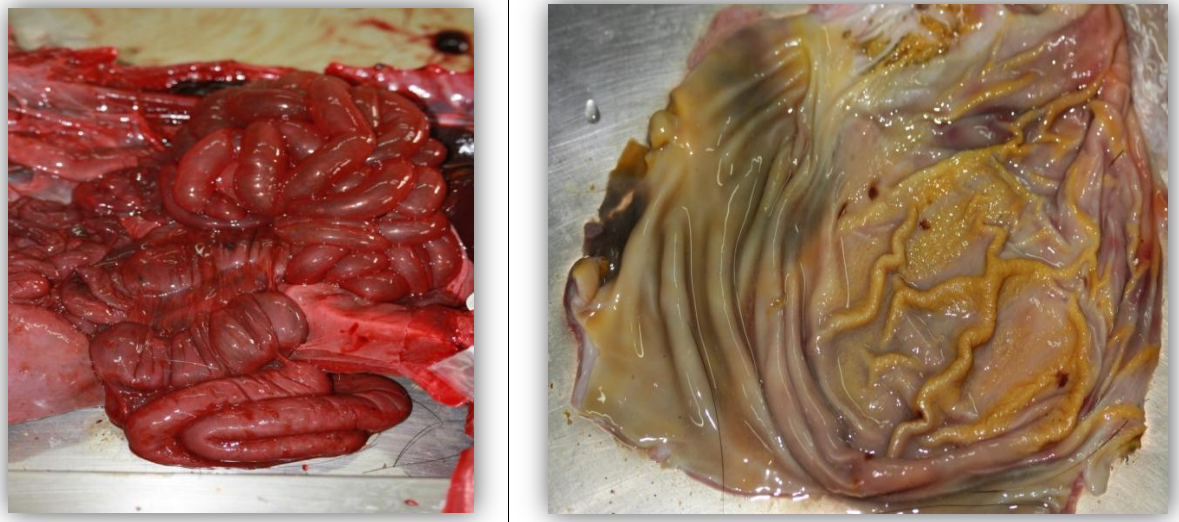
Graphs 20,21: Plots of *Mycoplasma* ELISA x Cecum contents (0-1) and Sex (Male=0, Female=1)

OTHER DISEASES, FINDINGS, LESIONS

Despite the fact that the animals were treated orally with the anticoccidial agent Toltrazuril at the moment of their capture and processing, enteritis signs and lesions were discovered in a number of piglets during necropsies. Lesions included small intestine hyperemia, necrotic regions, blood staining of perineal region and hemorrhage, intestinal lymph node enlargement and various types of diarrhea (figure 3). Specifically, 17.76% percent (27/152) of the animals necropsied had intestinal lesions, variable type of enteritis signs. Notably, by coding intestinal lesions as a nominal (0-1) variable and performing statistical analysis we found statistical difference in percentage of cecum contents of piglets grouped by intestinal lesions. Piglets with intestinal lesions had

statistically significant higher (U-Statistic=373.000, p=0.049) percentage of cecum content than the ones without lesions (Graph 22).

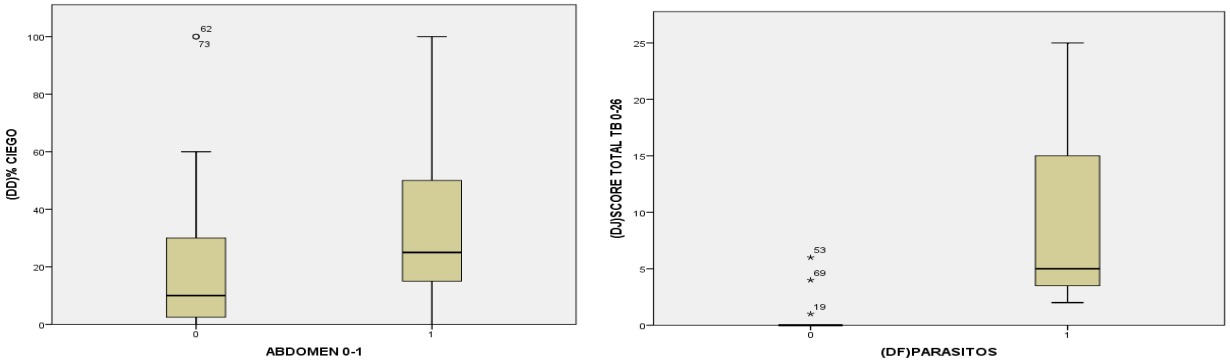
In addition, intestinal parasites were discovered inside the gastrointestinal tract, primarily nematodes, which were macroscopically identified as ascaridae parasites, or commonly known as large roundworms. These intestinal parasites were detected in 13.79% percent (12/87) of the animals.



<u>Figures 3,4</u>: 3. Hemorrhagic and dilated small intestine 4. Stomach with multiple dotted ulcers.

Again, after variable coding and performing stat tests, we found statistical difference between tuberculosis lesions scoring grouped by parasitosis (U-Statistic=3.0000 p=0.002), animals with intestinal parasitosis had significant higher TB scores than those without (Graph 23).

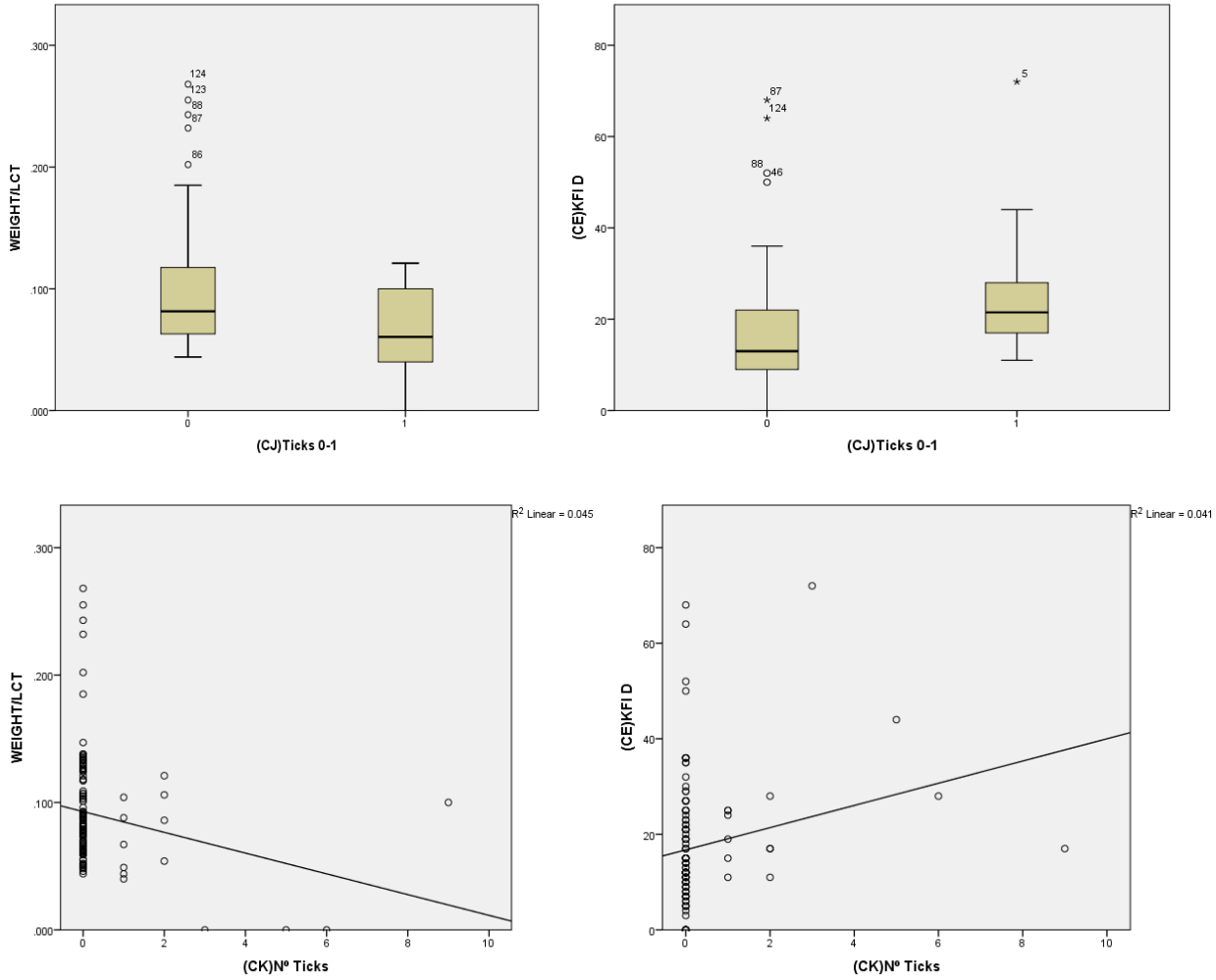
Yet another type of lesions which was discovered in the GI tract and monitored was gastric ulcers (figure 4), with a prevalence of 9.4% (5/53).



<u>Graphs 23,24:</u> 23. Percentage of cecum content x intestinal lesions 24. Intestinal Parasites x TB lesions scores

Finally, concerning parasites, there were also ticks detected in a number of animals, yet with a low prevalence of 12.09% (15/124). There was only one animal found with a very high tick count (counted more than 50 ticks). The rest had a relatively low count, in specific the infestations varied from nine to zero arthopodae.

There were two variables created from tick data, number of ticks (continuous), and tick presence/absence (nominal). Both these variables were found to statistically relate to kidney fat index and to a growth ratio (weight/total body length). Significant difference was found between kidney fat index (U-statistic=442.500, p=0.012) and weight/body length (U-statistic=473.500, p=0.023) when grouped by presence/absence of ticks (Graphs 25, 26), and also correlation was detected between number of ticks and the aforementioned variables (KFI: strength 0.234, p=0.01, Weight/total body length negative strength -0.206, p=0.023, Graphs 27, 28).



<u>Graphs 25-28:</u> Weight/total body length and kidney fat index grouped by ticks presence and correlation with tick number

TABLE 2: BIOMETRIC DATA VALUES

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
WEIGHT AT TIME OF DEATH	152	24.000	2.000	26.000	6.48289	3.946294	15.573
WEIGHT AT CAPTURE	365	12	3	15	7.51	2.168	4.700
TOTAL BODY LENGTH	151	65	51	116	68.67	11.479	131.770
HIND LIMB LENGTH	153	44	12	56	17.10	6.664	44.405
THORACIC PERIMETER	153	74	12	86	42.84	11.438	130.817
SPLEEN WEIGHT	173	231	3	234	38.13	33.764	1139.984
KIDNEY WEIGHT WITH FAT	138	173	8	181	28.28	21.232	450.788
KIDNEY WEIGHT NO FAT	151	118	8	126	23.16	14.899	221.975
RIGHT ADRENAL GLAND WEIGHT	96	24	0	24	1.29	2.374	5.638
SexF0M1	392	1	0	1	.51	.500	.250
KIDNEY FAT INDEX	138	427	0	427	20.21	37.223	1385.525
LUNG WEIGHT	129	458	54	512	184.71	87.483	7653.319
FAT INDEX	126	4	0	4	1.64	.986	.971
PERCENTAGE OF STOMACH CONTENTS	92	90	0	90	22.66	23.597	556.841
PRECENTAGE OF CECUM CONTENTS	84	100	0	100	23.21	24.735	611.833
WEIGHT LOSS	388	32.1400	-16.9000	15.2400	4.595974	5.0227136	25.228
WEIGHT/TOTAL BODY LENGTH	150	.268	.000	.268	.08938	.042043	.002
LUNG WEIGHT/BODY WEIGHT PERCENTAGE	65	30.1858	12.1519	42.3377	24.393139	7.2548435	52.633
WEIGHT/BODY LENGTH/HIND LIMB	129	.12	.02	.14	.0379	.01669	.000
Valid N (listwise)	31						

BIOMETRIC DATA RESULTS

In order to investigate health status, response to environmental traits, but also biological factors and response to management changes, a variety of biometric data was systematically recorded during piglet dissection. The list of biometric data along with their values is shown in table 2.

Starting from nominal variables, when grouping by sex, we found statistical difference in kidney fat index (U-statistic=1771.000, p=0.012) and weight loss (weight at

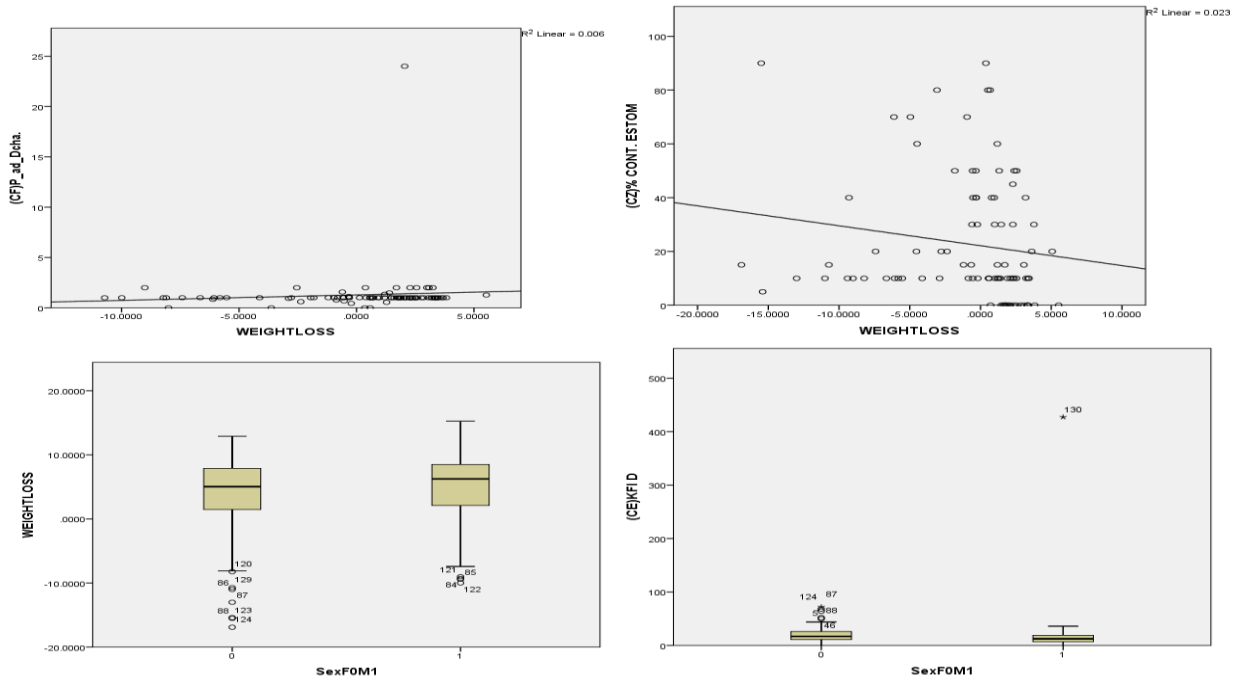
capture minus weight at death, U-statistic=16620, p=0.047) variables, with female having higher KFI and lower mean weight loss than male (Graphs 29, 30).

Concerning continuous variables significant results, weight at death was found to correlate negatively with all except one of the lung lobes consolidation percentage, and with the total consolidation index, as shown in table 3. Relationship between weight and total consolidation index has already been shown above in Graph 12.

Table 3: Weight x lung lobes consolidation percentages correlations

			Weight death	TOTALCONSOLIDATIONINDEX	CRI-INDEX 10%	MDI-INDEX 10%	DFI-INDEX* 25%	ACC-INDEX 10%	CRD-INDEX 10%	MDD-INDEX 10%	DFD-INDEX 25%
Spearman's rho	Weight death	Correlation Coefficient	1.000	-.283**	-.212*	-.309**	-.196*	-.283**	-.216*	-.184	-.205*
		Sig. (2-tailed)		.003	.026	.001	.040	.003	.022	.053	.031
		N	152	111	111	111	111	111	111	111	111

Relationship was also detected between right adrenal gland weight and weight loss (strength 0.260, p=0.011, Graph 31), and between percentage of stomach content and weight loss (negative strength -0.323, p=0.002, Graph 32).



Graphs 29-32: A: Weight loss and kidney fat index grouped by sex (Female=0, Male=1). B: Weight loss x Right adrenal gland weight and percentage of Stomach contents

DISCUSSION

A vital objective of the current study was to investigate disease status in wild boar of the youngest age, considering that currently a grey zone exists in this area, as most studies draw their data from hunting bag, which does not include piglets, which are not considered hunting targets.

As in modern swine industry, it seems that the way which hunting estates operate and organize for big game hunting in and around Central Spain, with high rates of animal aggregation at feeders with supplementary forage, and with high overall densities of animals, favor disease prevalence and circulation in general, a consequence which was verified also in the current study (Gortazar et al. 2006, Vicente et al. 2007).

Mycobacterium tuberculosis complex (MTC)

The high rates of MTC infection in our study (34% confirmed by culture, 37.5% positive by ELISA from total, 41.2% ELISA positive and 18.7% with TB-compatible lesions, among deceased and dissected piglets) agrees with the recent results of Vicente et al. 2013, who estimated a rate between 30-40% with TB lesions in wild boar piglets and weaners in Ciudad Real province, a location with similar management characteristics to ours. Yet, our results are significantly lower than those found by Diez-Delgado et al. 2014 in Montes de Toledo, South-central Spain, that reach as high as infection rates of 50% (measured by TB-compatible lesions) in piglets.

These results further strengthen the opinion that wild boar comes in contact with MTC from a very young age, but as a chronic infection, tuberculosis does not have a full- blown effect at this age group. The above is further supported by the fact that, while antibody rates and thus contact with the pathogen was relatively high, the mean TB-lesion score was found low (1.2 in a 0-26 scale).

Porcine circovirus type 2 (PCV2)

The PCV2 prevalence rates detected both by PCR and by ELISA (26% and 31% respectively) show that the specific virus is relatively widespread among piglets, in agreement with Vicente et al. (2004) results (38%), but somewhat lower than Boadella et al. 2012 (47%), and significantly lower than Diez-Delgado et al. 2014 (85%) rates.

Spleen weight has been established in deer as an immune strength indicator (Corbin et al. 2008). So, according to the above, its association with PCV2 results may be a factor which further strengthens the assumption made by Diez-Delgado et al. (2014),

that due to dichotomy in the humoral and cellular immune responses of swine, early PCV2 infection may indeed impair ability of wild boar piglets to respond to other infections, thus also including MTC.

Mycoplasma hyopneumoniae

Our PCR results of 18.2% seem to be in agreement with the results of Risco et al. (2014) who detected a 16.67% ELISA antibody prevalence in yearlings from Mid-Western Spain. With a mean lung consolidation percentage of 17%, it is somewhat clear that bacterial pneumonia is of great importance as a mortality contributor in this age group, at least in our case.

Taking into account that *M.hyopneumoniae* is considered as main cause of lung consolidation lesions, and summing with the infection prevalence of the bacteria in our case, we can conclude that *M.hyopneumoniae* played a major role leading the way towards broncho-pneumonia, thus maximizing death rates.

The above hypothesis is further supported by the higher lung weight rank in *M.hyopneumomniae* infected piglets, as it is a known fact that lungs with broncho-pneumonia lesions tend to have a higher weight (Morris et al. 1995). It is also a fact in domestic pig, as it was also discovered in our study, that enzootic pneumonia impedes development and weight gain (Maes et al. 1999), as lung consolidation percentage was associated not only with animal weight at time of death (as also every individual lung consolidation percentage was found to correlate with weight, with only a sole exception) but also with multiple development and weight gain indexes.

Other lesions

Besides tuberculosis and respiratory diseases, it is clear from the dissection results that miscellaneous factors contribute in the formation of such high mortality rates, as well. In specific, in our case, judging from GI lesions percentages (nearing 18%, despite the anticoccidial treatment), it seems that pathogens which reside in the GI tract (diarrheal complex pathogens) may also play a significant role in the above pathogenesis and should be further investigated (diagnostic tests etc.).

Also, the high prevalence of intestinal parasites (near 14%), together with statistical association of parasites with TB lesions, further complicates our scenario, as it seems that parasites also contribute to the emergence of disease and eventually death, directly via immunosuppresion, and also indirectly by promoting tuberculosis expansion.

Finally, high levels of stress, during period of capture and intensive management (artificial weaning) may contribute indirectly to mortality, by favoring disease invasion

and further lowering immunization capabilities, as statistical association between adrenal gland weight (a stress indicator) and weight loss was detected. Moreover, the detection of gastric ulcers (9.4%) ought to qualify as yet another indicator of high stress levels (Swaby et al. 2012, Amory et al. 2006).

Another interesting result is the association between hematomas, and both lung consolidation, and intensity of TB lesions, which raises the question, if this factor is associated and could be evaluated as a chronic stress indicator in farmed wild boar, as behavioral patterns have been experimentally correlated, and are currently used for that purpose in domestic swine (Munsterhjelm et al. 2013).

Pathogen interactions

According to our results, an association seems to exist between tuberculosis and *M.hyopneumoniae* occurrence. It seems that these pathogens interact with synergy in facilitating infection. To the best knowledge of the author, this is the first time which such an interaction is being witnessed, as Risco et al. (2014) who also conducted a similar study quite recently with the same pathogens did not discover a synergy between them.

Yet in the aforementioned study, the authors detected a synergistic effect similar to ours in the means of helminth infection, as *Metastrongylus* spp. was found to favor TB infection. Furthermore the authors speculate that this effect may be caused due to immunosuppresion from the parasitic infection. In our study, this assumption can be further strengthened as nematodes have been proved to produce immunosuppresion and favor tuberculosis in recent studies on African Buffalos (Ezenwa et al. 2011), and also humoral and cellular immune responses were successfully suppressed during in-vitro experiments using *Ascaris suum* extract, thus favoring establishment of *M.bovis* infection in mice (Ferreira et al. 1999).

CONCLUSIONS

We consider the first objective of our study has been accomplished, at least to an extent, as a variety of infectious agents were detected, further tested and identified as having a major or minor role in disease status of this age group, in semi-intensive management conditions.

To summarize, and also to address our second and third objective, it seems that pathogens which cause respiratory disease complex, together with intestinal nematodes and bacteria seem to play a major role in disease status and in mortality rates of wild boar piglets altogether. Specifically, there seems to be a complex relationship between infectious agents, as certain pathogens may weaken the piglet immune response, thus

allowing further establishment of other pathogens, such as tuberculosis, which does not seem to be a main contributor in mortality rates at this age group.

Also, a question further rises, whether capture and intensive management really benefits this age group, as indicators exist that high levels of stress are being induced, thus contributing to higher infection and death rates.

Yet, further studies should be designed, considering also statistical modeling, in order to better understand and map pathogen relationships and interactions.

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Phacochoerus africanus

2014