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Pathological and aetiological studies of multifocal interstitial nephritis in wasted pigs at slaughter

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Abstract

Multifocal interstitial nephritis in pigs has been associated with several infectious agents. The objective of the present study was to investigate several different potential infectious agents associated with "white-spotted" kidneys in pigs suffering from wasting at slaughter (aged 6–8 months). Twenty-nine case kidneys (with a "white-spotted" gross appearance) classified into 3 macroscopic lesional grades, and 15 control kidneys (lacking gross lesions), were obtained from a pig abattoir. Laboratory analyses to detect potential associations with the aforementioned pathological condition with *Leptospira* spp., porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), and bacteria, were carried out. Microscopically, interstitial nephritis with a lymphofollicular inflammatory pattern (follicular nephritis) was observed in both case and control kidneys, with a higher frequency seen in the former ones. No leptospires were identified, although antibodies to the Pomona and Bratislava serovars were detected. Some pyogenic bacteria were also isolated from both case and control kidneys. PCV2 nucleic acid was only detected in 1 case kidney. PRRSV antigen was not found in any tested sample. Some pigs were tested positive for PPV by serology. Apparently, none of the studied agents were specifically associated as being the potential cause of the renal lesions in the studied wasted pigs. The fact that these chronic lesions may have been the consequence of a previous infection with one of these studied microorganisms, or more, and eventually with other non-tested infectious agents during the growing-finishing period, cannot be ruled out.

Keywords: Interstitial nephritis; Pig; Wasting; Slaughter; Infectious agents

1. Introduction

Gross lesions of multifocal interstitial nephritis (MFIN), usually called "white spotted" kidneys, are one of the most common condemnation causes of pig kidneys at abattoirs (Drolet et al., 2002). Numerous studies have attempted to elucidate which infectious agents are involved in interstitial nephritis in apparently healthy pigs at slaughter, but results have considerably varied (Hunter et al., 1987; Jones et al., 1987; Baker et al., 1989; Chappel et al., 1992; Drolet et al., 2002; Boqvist et al., 2003).

For many years, one of the bacteria that has been traditionally associated with these lesions is *Leptospira interrogans* (Michna and Campbell, 1969; Jones et al., 1987; Baker et al., 1989), although most recent reports failed to demonstrate this association (Drolet et al., 2002; Boqvist et al., 2003). Leptospirosis is an infectious zoonosis caused by various serovars of *L. interrogans* sp., which affect a large number of wild and domestic species. Many serovars have been described to infect pigs, and the Pomona and

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Bratislava serovars are among the most common ones (Ellis and Thiermann, 1986; Perea et al., 1994; Boqvist et al., 2002).

Besides *Leptospira* spp., other infectious agents have been proposed as being aetiologically linked with interstitial nephritis, such as the porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV) and porcine adenovirus (Nietfeld and Leslie-Steen, 1993; Drolet and Dee, 1999; Drolet et al., 2002).

Nowadays, on the other hand, there are no published studies on ''white-spotted'' kidneys in pigs suffering from wasting at slaughter. These pigs show a lower growing rate, and they remain in the fattening units for longer periods than their apparently healthy batch mates. At slaughter, wasted pigs usually show chronic diseases and lesions that are sometimes the cause of total condemnation. In fact, a high incidence of kidney lesions was noted among the wasting pigs in relation to that seen in apparently healthy pigs (P. Jaro, Slaughterhouse Official Veterinary Inspector, Valencia, Spain, personal observation), although this information has not been published elsewhere.

Therefore, the purpose of the present work was to characterise the lesions associated to ''white spotted'' kidneys in wasted pigs at slaughter, and to investigate the potential role of several infectious agents (*Leptospira* spp. and other bacteria, PRRSV, PCV2 and PPV) and their relation with the renal lesions.

2. Materials and methods

2.1. Data from the abattoir

The studied pigs belonged to 11 different farms, all of which are located in Eastern Spain. The animals were sacrificed in a local abattoir in Valencia (Spain). Pigs were aged 6 to 8 months, with a mean carcass weight of 47.0 kg.

2.2. Sample collection

Forty-four kidneys from 44 different pigs suffering from wasting and sacrificed at slaughter were selected for the present study. The specific wasting causes were not recorded; animal selection was only based on the animal's clinical appearance. Twenty-nine kidneys with multifocal white spots on the cortical surface (case samples), and 15 kidneys with no evident gross lesions (control samples), were selected. Samples were taken over a 3-week period (once a week); 9 or 10 cases, and 5 control kidneys, were randomly collected from the total number of wasted pigs slaughtered on each sampling day.

Kidneys were pathologically classified from 0 to 3 following the macroscopic criteria proposed by Baker et al. (1989). The grading criteria were as follows: grade 0 (no gross lesions), grade 1 (less than 10 whitish foci between 2–5 mm in diameter), grade 2 (more than 10 whitish foci, or the presence of one white stain, or more, measuring less than 1 cm in diameter), grade 3 (renal cortical tissue completely covered by whitish foci or stains). Therefore, grade 0 corresponded to control kidneys while grades 1–3 corresponded to case renal samples. Tonsils from the selected pigs were also collected.

Kidney portions from each studied pig were placed in individual sterile plastic bags, which were refrigerated for subsequent bacteriological analyses. Other portions of renal tissues were placed in a tube containing liquid transport medium (TM), and were sent to the laboratory without refrigeration. Kidney and tonsil sections were fixed by immersion in 10% buffered formalin. A new set of sterile instruments was used for the sampling of each kidney.

A blood coagulum from the cardiac cavities of each studied pig was immediately (10 min after sacrifice) collected to obtain serum.

2.3. Histopathology, immunohistochemistry and in situ hybridisation

Tissue samples from kidneys and tonsils were maintained in formalin between 24 and 48 h, and were subsequently dehydrated and embedded in paraffin wax. Four micrometre thick sections were cut and stained following the haematoxylin–eosin (HE) and Warthin–Starry (silver stain) methods.

Two protocols were used to identify *L. interrogans* by immunohistochemistry (IHC) in kidney. The first, described by Nally et al. (2004), used a primary rabbit antibody specific for outer membrane vesicles of the *L. interrogans* serovar Copenhageni SPFL isolates RJ15958 at a dilution of 1:500. The second protocol was described by Ramos et al. (1991), and was carried out to identify the following serovars: Icterohaemorrhagiae, Pomona, Canicola, Bratislava, Hardjo and Grippotyphosa. The primary antibody came from the National Leptospirosis Centre (National Animal Disease Center, Ames, IA, USA), and was used at a dilution of 1:800. Both *Leptospira* spp. IHC methods were performed on the kidneys of all studied animals.

An IHC to detect the PRRSV antigen was performed using the primary antibody SDOW-17-A (Rural Technologies Inc., SD, USA) following the protocol described by Segalés et al. (1999). PRRSV IHC was performed on the tonsils and kidneys of all studied animals.

An in situ hybridisation (ISH) to identify nucleic acid of PCV2 was carried out following the protocol described by Rosell et al. (1999). ISH was also performed on the tonsils and kidneys of all studied animals.

Appropriate positive and negative control tissues were included to ensure the proper assay performance in the IHC and ISH methods.

2.4. Bacteriology

Samples for the bacteriological analyses were immediately transported to the laboratory.