Leptospira interrogans serovar Pomona infection associated with carcass condemnation of swine at slaughter

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Summary

This report describes a clinical case of *Leptospira interrogans* serovar Pomona infection in finishing pigs, associated with multifocal interstitial nephritis that resulted in carcass condemnation at slaughter. Clinical signs of leptospirosis were not observed. On gross examination, kidneys varied from slightly shrunken to 1.5 times normal size, with pale mottling extending through the cortex and into the medulla. Microscopic examination revealed tubulointerstitial nephritis with lymphoid nodules in affected kidneys. Renal lymph nodes were three to 10 times normal size, often having a cystic appearance. Diagnostic results indicated that, in this herd, serovar Pomona infection had been introduced into the finishing barns with resulting widespread endemic infection in these buildings. Clinical management included antibiotic therapy for the grower and finisher pig inventory, site depopulation with cleaning and disinfection of the facilities, and strict rodent control.

Keywords: swine, finishing pigs, *Leptospira interrogans* serovar Pomona, carcass condemnation, focal interstitial nephritis

Received: January 13, 2005
Accepted: March 28, 2005

Resumen – Infección por *Leptospira interrogans* serovariedad Pomona asociada con la eliminación de canales de cerdos en el rastro

Este reporte describe un caso clínico de infección por *Leptospira interrogans* serovariedad Pomona en cerdos de finalización, asociada a nefritis intersticial multifocal que resultó en la eliminación de canales en el rastro. En este caso no se observaron signos clínicos de leptospirosis. En el examen macroscópico, el tamaño de los riñones variaron de ligeramente reducidos a 1.5 veces su tamaño normal y con manchas pálidas extendiéndose de la corteza a la médula. Un examen microscópico reveló una nefritis tubulointersticial con nódulos linfáticos en los riñones afectados. Los nódulos linfáticos renales presentaron un tamaño tres a 10 veces mayores al tamaño normal, frecuentemente con una apariencia cística. Los resultados diagnósticos indicaron que, en este hato, la infección por serovariedad Pomona había sido introducida a la finalización provocando una infección endémica en estas instalaciones. El manejo clínico incluyó terapia antibiótica para el inventario de cerdos de crecimiento y finalización, despoblación del sitio, limpieza y desinfección de las instalaciones y estricto control de roedores.

Résumé – Infection à *Leptospira interrogans* serovar Pomona associée à la condamnation de carcasses de porc à l'abattoir

Le présent rapport fait état d'un cas clinique d'infection à *Leptospira interrogans* serovar Pomona chez des porcs en engraissement, associé à une néphrite interstitielle multifocale entraînant la condamnation de carcasses à l'abattoir. Bien qu'aucun signe clinique de leptospirose n'ait été observé lors de l'examen macroscopique des reins, la grosseur de ces derniers variait de légèrement atrophié à 1.5 fois la taille normale et de pâles mouchetures s'étendant du cortex jusque dans la médulla ont été notées. L'examen microscopique a révélé une néphrite tubulo-insterstitielle avec des nodules lymphoïdes dans les reins affectés. Les nœuds lymphatiques rénaux étaient de trois à 10 fois leur taille normale, ayant souvent une apparence cystique. Les résultats indiquent que dans ce troupeau, l'infection associée à serovar Pomona a été introduite dans les bâtiments de finition avec comme résultat une infection endémique disséminée chez les animaux dans ces bâtiments. La gestion clinique de ce cas incluait une antibiothérapie pour les porcs en engraissement et en finition, une dépopulation du site avec nettoyage et désinfection des lieux, et une surveillance accrue de la vermine.

Leptospirosis is a worldwide zoonotic disease caused by infection with any of the pathogenic serovars of the spirochete bacterium *Leptospira interrogans*.[1] Within a geographic region, a relatively small number of leptospiral serovars are prevalent.[1] Serovar *Pomona* has been the most common serovar isolated from pigs worldwide.[1] Infection may exist in individuals or a herd without clinical signs,[2] and once serovar Pomona has been introduced into a pig population, a high prevalence of infection is established.[1] The primary signs of chronic leptospirosis,

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This article is available online at http://www.aasv.org/shap.html.

Carpenter JA, Scorgie A, Josephson G. *Leptospira interrogans* serovar Pomona infection associated with carcass condemnation of swine at slaughter. *J Swine Health Prod.* 2006;14(3):145–148.

particularly serovar Pomona infection, are abortion, infertility, birth of weak or dead piglets,[1] and chronic interstitial nephritis.[3] The presence of leptospires in the kidneys of swine at slaughter has been associated with lesions of multifocal interstitial nephritis (MFIN).[3] These lesions, known as white spots, consist of scattered small gray foci, often surrounded by a ring of hyperemia. Microscopically, these are lesions of progressive focal interstitial nephritis.[1]

Other causes of interstitial nephritis in swine include bacteria such as *Escherichia coli*, staphylococci, and streptococci; however, a study conducted in South Africa demonstrated that the lesions caused by these organisms were extremely mild, and were not associated with macroscopic lesions described as white spots (ie, MFIN).[3]

Porcine dermatitis and nephropathy syndrome (PDNS), a vascular disease affecting weaned pigs, growing-finishing pigs, and, less commonly, breeding animals,[4] may be included as a differential diagnosis for kidney lesions in swine. Grossly, either necrotizing skin lesions occur, primarily on the hind limbs and perineal area, or the kidneys are swollen and pale with generalized cortical petechiae, or both.[5] Microscopically, there is systemic necrotizing vasculitis with necrotizing and fibrinous glomerulonephritis.[5]

This report describes *L interrogans* serovar Pomona infection in finishing pigs that resulted in condemnation of carcasses at slaughter. The treatment plan instituted to address the problem is outlined.

Herd description

The affected production system was a multiple-site, 900-sow farrow-to-finish operation, including two 450-sow operations (Sites H and L, approximately 1 km apart) with all in-all out farrowing and stage-one nursery rooms. Site L also had a continuous-flow stage-two nursery and six shelters for grower pigs (operated all in-all out by shelter).

Three finishing locations were included in this system: Sites M and C (45 km apart and each approximately 30 km from Sites H and L) and Site P (approximately 2 km from site H). Site M had a continuous-flow grower barn connected by an alleyway to two continuous-flow finishing barns, each containing two large pens. A water trough ran through both pens in each finishing barn, and an automatic hog sorter was located in one barn. Site C had a continuous-flow, partially-slatted barn. Site P had a barn housing one large group of pigs and had an automatic sorter.

The production system multiplied its gilts internally and received semen from its own boar stud. The herd was serologically negative for porcine reproductive and respiratory syndrome (PRRS) virus and positive for *Mycoplasma hyopneumoniae*. Sows were vaccinated against *Leptospira interrogans* serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona; porcine parvovirus (PPV); and *Erysipelothrix rhusiopathiae*.

Pigs weighing 12 to 15 kg were moved from the two stage-one nurseries into the stage-two nursery located on Site L. Pigs weighing 20 to 25 kg were moved from the stage-two nursery to one of the shelters at Site L or to Sites C or M, depending on space availability at each location. Pigs were housed in the shelters until they reached 70 to 90 kg body weight and were then moved to Site P. At Site C, pigs were housed in the same barn during the grower and finisher phases. At Site M, pigs were housed in the grower barn until they reached 75 kg and were then moved into a finishing barn.

A feed mill at Site L made gestation and nursery feed for both sow operations, as well as grower feed for pigs in the shelters. Lactation feed was purchased from a commercial feed mill for both sow herds. Waste cereal products (ie, sweetened commercial breakfast cereal) and dog food were collected and stored in a commodity barn located at Site P. Feed was made at Site P, using these ingredients, for the finishing pigs at Sites P, C, and M.

Water quality and quantity was a problem in this system, and as a result, waste water was purchased from a rice-processing company and stored in large tanks at all sites except Site C, which did not receive rice water. Eight to ten thousand liters of rice water were picked up daily (5 days per week).

Case description

Between January 1 and August 28, 2002, a total of 68 carcasses from this production system were condemned due to glomerulonephritis. In some cases, the kidneys appeared grossly normal, but the carcasses were condemned because of enlarged renal lymph nodes. No sick pigs were detected prior to marketing. Weekly carcass condemnation numbers rose from five early in August to 12 on August 21. On August 29, the herd veterinarian arranged for submission of six representative kidneys and renal lymph nodes from pigs slaughtered that week to the Animal Health Laboratory (AHL), Guelph, Ontario, for diagnostic testing.

Throughout September, five to 12 pigs per week were condemned for glomerulonephritis. As the pigs from the three finishing sites had been marketed under the same tattoo number, it was unknown if all finishing barns were affected or just one; therefore, in mid-September, each finishing barn was assigned a separate tattoo number.

Clinical signs

On September 13, the herd veterinarian visited Sites L, M, and P. At that time, the pigs in the shelters at Site L were ready to be moved to Site P. The grower pigs at Site M had been in the grower barn for 1 week. The pigs in one Site M finishing barn were 4 weeks away from market, while in the other barn, the pigs were market age. Pigs in the finishing barns at Sites M and P were being treated with lincomycin in the drinking water for a *M hyopneumoniae*-related respiratory problem. Variability in growth was evident in all barns, but it was most pronounced at Site M, where the pigs appeared "slab-sided" and poorly muscled.

Two representative pigs from a Site M finishing barn were euthanized, and tissue samples (kidney, heart, spleen, lung, and intestine) were collected in formalin. Grossly, the kidneys from both pigs were enlarged and appeared mottled. A total of 64 blood samples were drawn: 11 from finishing pigs at Site P, 11 from grower pigs in the shelters at Site L, and 12 from grower pigs and 30 from finishing pigs at Site M. Blood and tissue samples were submitted to the AHL. A water sample from the well at Site M was submitted to GAP Microbial Services, London, Ontario, to culture for *Leptospira* species. As the rice-water storage tank at Site M was empty, a sample could not be collected.

Laboratory results

Lesions varying in severity and duration were present in all six kidneys submitted on August 29. On gross examination, three

kidneys were swollen, edematous, and pale, with randomly scattered petechial hemorrhages on the surface, with or without fibrin tags associated with the hemorrhagic foci. The remaining kidneys were randomly mottled, with variable pale foci ranging from 2 to 5 mm in diameter on a background ranging from wine to normal kidney color. Lesions extended to the medulla as wedge-shaped areas or occasionally as diffuse pale areas. Differential diagnoses included PDNS and leptospirosis.

One of two pools of urine aspirated from kidneys submitted at slaughter was positive on a polymerase chain reaction (PCR) test for pathogenic leptospiral serovars (validated only for dog urine). Serovars included were Autumnalis, Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona. Kidneys from slaughtered pigs were negative for PPV by fluorescent antibody test and positive for porcine circovirus type 2 (PCV2) using PCR and restriction fragment length polymorphism (RFLP) typing. Culture of kidney and lymph node resulted in no bacterial growth.

Histopathological examination of submitted kidneys revealed multiple foci of lymphohistiocytic and plasma cells, occasionally as nodules, but more often as diffuse foci in the cortex. A more widespread but less severe reaction was present in the medulla. Histological diagnosis was multifocal interstitial nephritis.

The 64 blood samples were tested using the microscopic agglutination test (MAT): samples were reacted with live antigen suspensions of Leptospiral serovars Pomona, Grippotyphosa, and Bratislava. All 30 serum samples from finishing pigs at Site M were positive for serovar Pomona, with titers ranging from 1:640 to 1:20,480. Titers of < 1:80 are considered negative, 1:80 to 1:160 are suspicious, and > 1:160 are positive (AHL). Serum samples from grower pigs in the shelters at Site L, grower pigs at Site M, and finishing pigs at Site P were negative for serovar Pomona. All serum samples were negative for serovar Grippotyphosa but suspicious or positive for serovar Bratislava, with higher titers (up to 1:20,480) in the finishing pigs at Site M. *Leptospira* serovars were not cultured from the water sample. Kidneys from both the slaughter pigs and the two euthanized pigs were positive for *Leptospira* species using immunofluorescent stains. Histopathological examination of the slaughter pig kidneys revealed tubulointerstitial nephritis, with lymphoid nodules in affected kidneys. Renal lymph nodes were three to 10 times normal size, often having a cystic appearance. Warthin-Starry stain identified spirochetal organisms within nephric tubules. Final diagnosis in the finishing pigs at Site M was interstitial nephritis due to serovar Pomona.

Treatment and outcome

Treatment of the finishing pigs at Site M began on September 19, after the serology results were known. The finishing pigs were treated for 5 days with water-soluble tetracycline hydrochloride, then medication was withdrawn for 5 days and market hogs were shipped. The remaining pigs received medication in the drinking water for 5 additional days. In each finishing barn, the slab-sided pigs were housed in a hospital pen and treated with antibiotics by injection. Heavier pigs received injections of oxytetracycline (6.6 mg per kg once daily) for 3 days. Lighter pigs received injections of both long-acting oxytetracycline (20 mg per kg) and streptomycin (25 mg per kg) on two occasions 2 weeks apart. The pigs in the grower barn at Site M received chlortetracycline in the feed at 550 g per tonne (22 mg per kg body weight once daily). Rice-water feeding was halted in all barns. There was a good response to treatment: 3 weeks after the last treatment, only one pig was identified with renal lesions at slaughter.

Feeder pigs were sold from the production system to allow for depopulation of Site M. The barns were washed, disinfected, and left empty for 1 week. The pits were pumped out, the rice water tank was flushed, and an aggressive rodent control program was instituted. To date, there have been no further indications of leptospirosis, including carcass condemnations.

Discussion

In this production system, clinical signs of leptospirosis were not reported by the owner, and therefore the diagnosis of leptospirosis in the finishing pigs at Site M was based on laboratory results. Diagnostic tests for leptospirosis include assays for serum antibody and assays to detect the organism in tissues or body fluids.[1] The most commonly used diagnostic test for leptospirosis is the MAT, which is the reference test for serological diagnosis of *Leptospira* serovars.[1,6] The test result is reported as a titer representing the highest dilution of serum that agglutinates at least 50% of the living leptospires.[6] The MAT is used primarily as a herd test: at least 10 animals, or 10% of the herd, should be tested.[1] Titers due to infection with serovars other than Bratislava tend to be 1:800 or greater.[7] The immunogenicity of different leptospiral serovars varies greatly, so some serovars induce much higher MAT titers than others.[8] As serovar Pomona is among the more immunogenic serovars, infection usually induces high titers in unvaccinated animals.[8] Vaccination and cross-reacting antibodies may interfere with interpretation of serologic results.[7] In a pig infected with a single serovar, the MAT is likely to detect antibodies against more than one serovar, but usually the titers are considerably higher against the infecting serovar.[7] In this case, serum samples from all 30 finishing pigs tested at Site M were positive for serovar Pomona using the MAT, with titers ranging from 1:640 to 1:20,480, and titers against serovar Bratislava were interpreted as cross-reactions to the serovar Pomona infection.

Histopathology with the use of silver stains is a useful technique to identify leptospires in tissue; however, the infecting serovar cannot be determined.[7] In this case, Warthin-Starry staining techniques identified spirochetal organisms in tubular cells in the kidneys of the slaughter pigs. Demonstration of leptospires in the kidney or urine, in the absence of evidence of generalized infection, is diagnostic of chronic infection.[1] Immunohistochemical testing using immunofluorescent stains showed clusters of spirochetal organisms in renal tubular lumens that stained with antiserum to *Leptospira* species. This test depends on the number of organisms present (ie, if only small numbers are present, the result might be a false-negative) and provides no information as to the infecting serovar.[1] Urine aspirated from submitted kidneys was PCR-positive for one of the group of pathogenic leptospiral serovars identified by the test, which is limited by its inability to identify the infecting serovar.[6]

The differential diagnoses in this case included PDNS and leptospirosis. Porcine dermatitis and nephropathy syndrome is an immune-mediated vascular disease with a strong and growing link to PCV2.[9,10] Antigen of PCV2 has been found in association with kidney lesions of affected pigs.[11] Cofactors

such as viruses (PRRS virus, swine influenza virus and PPV), and bacteria (*M hyopneumoniae* and *Haemophilus parasuis*), along with both immunosuppression and immunostimulation, have been suggested as necessary for the development of PCV2-associated disease, in that they potentiate replication of PCV2 (S. Krakowka, written communication, 2005).

In this case, a PCR test for PCV2 was conducted on kidneys from the slaughter pigs. The RFLP cut pattern was 4-2-2, identifying the virus as a pathogenic type 2 circovirus. However, identification of PCV2 by PCR merely indicates the presence of the virus and does not equate it with disease. The presence and location of the spirochetal organisms identified in renal tissue by Warthin Starry staining is highly suggestive of leptospiral infection. Immunohistochemical staining identified these as *Leptospira* species. This, plus the markedly elevated serovar Pomona MAT serum titres, and the elimination of the problem after antimicrobial therapy, give further credence to the diagnosis of serovar Pomona-induced nephritis.[12]

Different *L interrogans* serovars are prevalent in different geographical areas and are associated with one or more maintenance hosts that serve as reservoirs of infection, in which the disease is maintained by chronic infection of the renal tubules.[13] A maintenance host is defined as a species in which infection is endemic and is usually transferred from animal to animal by direct contact.[6] Pigs may be infected by serovars maintained by other pigs or serovars maintained by other animal species.[13] Urine is the chief source of contamination, and infected pigs may pass leptospires in the urine for long periods of time.[2] Maintenance hosts for serovar Pomona include dairy cattle, pigs, sheep,[6] and skunks.[1] Not all strains of serovar Pomona are adapted to pigs, but have rodent hosts.[13] Animals may be maintenance hosts of some serovars and incidental hosts of others that may cause severe or fatal disease.[6] Waterborne transmission has occurred in humans, and cases have been documented where point contamination of water supplies has resulted in several outbreaks of leptospirosis.[6]

Infection may be introduced into a susceptible herd by three possible routes: introduction of infected stock, exposure to a contaminated environment, or contact with an alternative infected animal vector.[1] The original source of the infection at Site M was not identified. The grower pigs at Sites L and M and the finishing pigs at site P were serologically negative for serovar Pomona (titers < 1:40), and water from the well at Site M was culture-negative for *Leptospira* species. It was concluded that, on Site M, serovar Pomona infection had been introduced into the finishing barns, and the infection had become widespread and endemic.

Control of leptospirosis depends on the combined use of antibiotics, vaccination, and management.[13] The control strategies used in this case included antibiotic treatment, site depopulation with cleaning and disinfection of the facilities, and strict rodent control. Vaccination was not considered, as it reduces the prevalence of infection in a herd without eliminating the infection.[1] Similarly, antibiotics alone will neither eliminate pig-maintained leptospiral infections from individual carrier animals nor control infection in herds.[1] In this case, the antibiotic treatment did succeed in reducing carcass condemnations of the infected pigs at slaughter. A critical factor in the control of leptospirosis in swine is interruption of transmission from an infected pig or other host,[1] and this was accomplished by site depopulation with cleaning and disinfection of the facilities.

Implications

- *L interrogans* serovar Pomona infection may cause multifocal interstitial nephritis in finishing pigs, resulting in carcass condemnation, without clinical signs of infection in the herd.
- Pigs in different barns on the same site may not all be infected with serovar Pomona, depending on the source of infection.
- A combination of antibiotic treatment, depopulation, and environmental decontamination is an effective control strategy for serovar Pomona infection in finishing pigs.

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