Systemic porcine salmonellosis: A potential zoonosis and cause of mortality in smallholder pig farm in Kenya

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SUMMARY

Salmonella enterica serovar *Typhimurium* and *Cholerasuis* are potentially zonootic pathogens that cause porcine salmonellosis; a disease associated with economic losses worldwide. Presence of this disease in pigs in Kenya is largely unknown. Two, 11-week old pig carcasses presented for necropsy to the Department of Veterinary Pathology, Microbiology and Parasitology were used in this study. Clinically, the pigs were depressed and developed yellowish diarrhea that was refractory to antimicrobial treatment. Systematic necropsy was conducted on the carcasses. Lung and colon samples were collected aseptically for bacteriology analysis. Other samples collected were lymphoid tissues, lungs, heart, liver, kidneys and gastrointestinal tract which were fixed in 10% formalin and processed routinely for histopathlogy examinations. *Salmonella enterica* serovar was isolated from lungs and colon. At post mortem, the significant findings included: emaciation and cyanosis of extremities, increased fluids in body cavities, generalized fat atrophy, enlarged lymphoid organs, congested and edematous lungs, epicardial hemorrhages, red renal infarct and miliary white foci randomly distributed in the liver. Histopathological examination revealed presence of macrophages in lymphoid tissue sinuses, pulmonary edema, infiltration of inter-alveolar septae by mononuclear cells, myocardial thrombosis and necrosis, glomerulonephritis, tubular nephrosis and multifocal areas of coagulative hepatic necrosis. In conclusion, the report documents the occurrence of systemic salmonellosis that resulted in mortality of the two pigs. The disease might be more widespread, and the herd status and the serovars involved in Kenya should be established.

Key words: porcine salmonellosis, zoonosis, pigs, Kenya

INTRODUCTION

Salmonellosis caused by *Salmonella enterica* serovar *Typhimurium* and *Cholerasuis* occurs most frequently in intensively reared pigs farms, possibly in extensive production systems in many countries (Griffin *et al.*, 2006). The infection is acquired through ingestion of feed or water contaminated by fecal material harboring the organisms. The bacteria then colonize the intestines by host-specific colonization factors to cause enterocolitis and diarrhea (Morgan *et al.*, 2004, Carnell *et al.*, 2007). Some bacteria transit through the intestinal wall to cause systemic disease (Pullinger et al., 2007). Incidentally, none of these two forms of disease have been confirmed in pigs in Kenya.

Salmonella enterica serovar *Typhimurium*; a widely reported pathogen of pigs in Europe (Penrith *et al.*, 2004), induces an enterocolitis in weaned pigs. An early intestinal inflammation is characterized by

neutrophil recruitment and transmigration across the epithelium (Balaji *et al.*, 2000). Cholera-like and shiga-like toxins produced by this serovar not only induce increased chloride secretion and decreased sodium resorption leading to diarrhea, but also cause toxic effect on epithelial cells. At the same time, endotoxins that are produced locally induce microvascular thrombosis and endothelial necrosis in sub mucosa and lamina propria that is expressed as ischemic lesions in the mucosa (Murray, 1986, Griffith *et al.*, 2006). On the other-hand, Salmonella enterica serovar Cholerasuis, often reported in pigs in United States (Penrith *et al.*, 2004), is highly invasive and manifests as systemic syndrome (Chui *et al.*, 2004). Massive liberation of endotoxins, exotoxins and peptide fragments in circulation cause septicemia that rapidly progress to intravascular coagulaopathy and shock if untreated (Cheville, 2006). Animals surviving this acute episode die of pneumonia, hepatitis, enterocolitis and rarely, menengoencephalitis (Griffith *et al.*, 2006).

In a clinical survey conducted in smallholder farms in Kikuyu region, Kenya, a crude mortality of 3.8% obtained in grower pigs was attributed to undifferentiated diarrhea (Wabacha *et al.*, 2004). Similarly, in a 10 year retrospective study on post mortem records, 27.7% (74/267) and 5.6% (15/267) pigs died of diarrhoea and septicemia, respectively (Karanja *et al.*, 2005). In all these cases, the specific causes were not determined, yet it is acknowledged that the majority of the smallholder farms operated in unhygienic conditions that are conducive to many septicemic and gastrointestinal problems (wabacha *et al.*, 2004). This report therefore aimed at documenting a confirmed outbreak of systemic salmonellosis in a smallholder farm in Nairobi region, Kenya.

MATERIALS AND METHODS

Two, 11-week old pigs from a flock of 14 pigs situated in Nairobi Province, Kenya were presented to the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi for necropsy in 2008. The pigs had been weaned at 8 weeks, developed a yellowish watery diarrhea. On two occasions, these pigs had been treated using antimicrobial drugs, but each time the drugs were withdrawn, relapses occurred. Systematic necropsy was conducted on the 2 carcasses submitted and lung tissue and colon contents were collected aseptically for bacteriology.

Bacterial isolation and characterization was conducted as described in Bergy's Manual of systemic bacteriology (Brenner, 1994). Briefly, 5 grams of colon contents and ground lung tissue were inoculated into tetrathionate broth, incubated at 37 ºC for 24 hours. A loopful of the broth was streaked onto MacConkey agar and incubated. Pale, non-lactose fermenter colonies were gram-stained and used in biochemical characterization.

Other samples were taken from various body organs and in particular the lungs, lymph nodes, spleen, liver, kidney and gastrointestinal tract for histopathology. The latter were fixed in 10% formalin, embedded in paraffin wax and processed routinely. Histological sections obtained were stained using hematoxylin and eosin before being examined with a light microscopic.

RESULTS

The two carcasses were emaciated and had bluish-red discoloration of the ears, feet and ventral abdomen. In one, straw-coloured fluid in the peritoneum, thoracic cavity and pericardial sac was found while in the other, the fluid was yellow in color while carcass was slightly icteric. Both had generalized fat atrophy and skeletal muscles were pale. The mesenteric lymph nodes were markedly enlarged and on cutting, they appeared wet. Microscopically, there was increased number of macrophages in the medullary sinuses. The spleen was also enlarged and congested and at histology, depletion of follicles and increased number of macrophages was a prominent feature.

The liver was enlarged, brown-orange in color and white foci measuring 0.5-1.0 mm were distributed randomly on the capsular surface and on the cut surface of the parenchyma. Microscopically, these foci comprised areas of coagulative hepatic necrosis mainly in peri-acinar areas. The kupffer cells were many and prominent. Lymphocytic cellular infiltrations around the portal triads were randomly distributed throughout the liver (Figure 1).

Kidneys in one case had a large red infarct in the cortex while the other appeared pale. Microscopically, there was increased cellularity of almost all the glomeruli mainly due to mononuclear cells. Many mononuclear cells were found in the interstitium between the renal tubules in the cortex. Tubular nephrosis was observed in both cases (Figure 2).

The lung tissues were heavily congested. Frothy exudates were found in the airways and interlobular spaces. Microscopically, heavy congestion and edema fluid were found. Almost all alveoli had eosinophilic staining exudates. In addition, a mononuclear cellular response was observed in inter-alveolar septae.

In both cases, the heart appeared more rounded and the myocardium was pale. Splashes of hemorrhages were observed in the epicardium, especially, in the ventricles. Microscopically, fibrinoid thrombi were found in arterioles serving areas with coagulative myocardial necrosis. A gram-negative cocco-bacilli that reduced nitrate to nitrite, produced hydrogen sulphide, fermented glucose and maltose and not sucrose or lactose was isolated from the lung tissue and colon contents of the two animals. This *Salmonella enterica* serovar isolate was also indole and urease negative.

DISCUSSION

A diagnosis of systemic salmonellosis was made on the two carcasses based on a combination of clinical history, post mortem findings and isolation of Salmonella enterica serovar from the lung tissue and the colon contents; a diagnostic method recommended for this disease (Penrith *et al*., 2004, Griffith *et al*., 2006). Though serotyping was not done in this study, isolation of any of the two serovars; Typhimurium or Cholerasuis, from the 2 cases have far-reaching implications. Both serovars have zoonotic potential as human salmonellosis has been traced to pork products (Penrith *et al.*, 2004, Stevens *et al.*, 2009). These serovars are also capable of causing porcine salmonellosis, a disease of significant economic importance worldwide (Penrith *et al.*, 2004, Griffin *et al.*, 2006).

In this study, the affected pigs died in spite of antimicrobial treatment; a feature that has been attributed to relapsing infection. It has been shown that *Salmonella enterica*

serovars may develop multiple antibiotic resistance (Baggensen and Aarestrup, 1998) and that antibiotic treatment only reduce bacterial load, but after drug withdrawal, bacteria grow in systemic tissues (Chui *et al.*, 2004, Griffin *et al.*, 2011). The disease in this outbreak might have been triggered by weaning as stress associated with this event results in increased faecal excretion of serovar *Typhimurium* in pigs and reactivation of asymptomatic infections (Isaacson *et al.*, 1999, Callaway *et al.*, 2006).

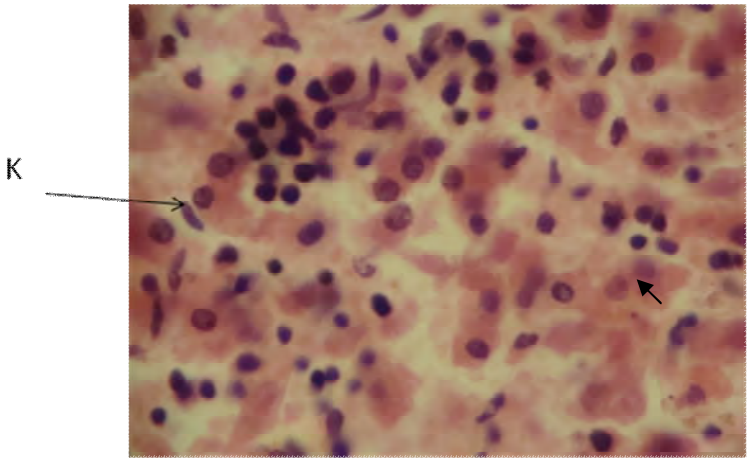

Figure 1. Histological section of liver from a piglet diagnosed as systemic salmonellosis, showing mononuclear cells infiltrating into hepatic parenchyma (arrows) and prominent kupffer cells (K). H & E X 400.

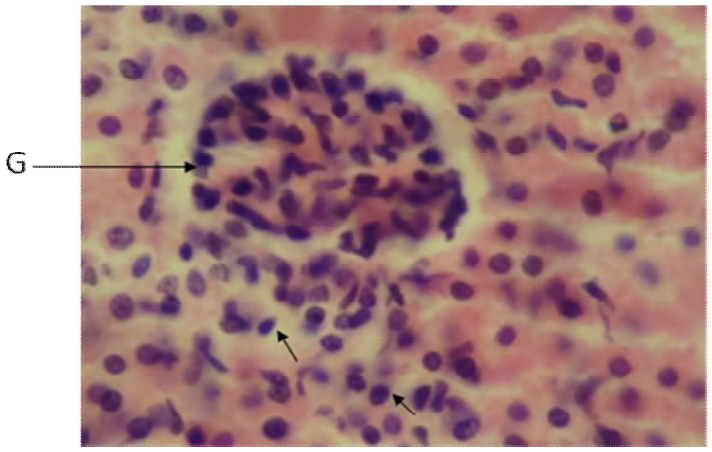

Figure 2. Histological section of the kidney from a piglet diagnosed as systemic salmonellosis showing mononuclear cells infiltrating into glomerulus (G) and in the surrounding interstitium (arrows). H & E X 400.

The post mortem finding observed in this study namely; cyanosis of extremities, epicardial hemorrhages, pulmonary edema and renal infarcts are commonly observed in septicemic syndromes (Cheville, 2006). The microthrombi observed in heart and kidney may also indicate effects of endotoxins and bacteria fimbriae on the

endothelium; a feature indicative of septicemia. The miliary white foci reminiscent of "typhoid nodules" and comprising areas of coagulative necrosis and increased number of histiocytes, high number of macrophages in medullary sinuses of lymph nodes and glomerulonephritis observed in this study are similar to those reported in systemic salmonellosis (Penrith *et al.*, 2004, Griffith *et al.*, 2006).

In the gastrointestinasl tract, no significant lesions were observed. As observed elsewhere, some bacteria transit through the intestinal wall to cause systemic disease and enterocolitis appear secondarily (Griffith *et al.*, 2006, Pullinger *et al.*, 2007). Emaciation on the other-hand indicates a prolonged disease characterized by anorexia and high levels of toxin in tissues (Cheville, 2006).

In conclusion, the report documents the occurrence of systemic salmonellosis that resulted in mortality in a small-holder pig farm. The disease might be more widespread in small-holder farms in Kenya, and therefore, further studies are required to determine the status of the pig herds as well as the serovars involved.

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