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Management of Outbreak of Salmonellosis in a Pig Farm Revealed Human Source of Infection in Cameroon

Abel Wade*

Laboratoire National Veterinaire (LANAVET) Annex Yaounde, Cameroon

*Corresponding author: Abel Wade, Laboratoire National Veterinaire (LANAVET) Annex Yaounde, Cameroon



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ABSTRACT

Food-borne Zoonoses such as salmonellosis are major public health problem worldwide. In this study, we described the management of an outbreak of salmonellosis in a pig farm in the South region of Cameroon. Laboratory investigation based on PCR, isolation & identification and antibiogram tests indicated that the Salmonella strain was sensitive to gentamycin and neomycin, but resistant to tetracycline and penicillin. Neomycin was administered to the pigs while gentamycin was used to treat the farmer itself. From our findings and investigations, the source of infection could be from the man in charge of feeding those animals. This calls for a strong collaboration among One Health sectors for a management of zoonotic diseases.

Keywords: Salmonellosis; Pigs; Human; Animal; One Health

Introduction

Salmonellosis is a zoonotic food-borne bacterial disease caused by Salmonella sp. Over 2,500 serotypes (serovars) of Salmonella are known to date [1]. The bacteria are generally transmitted between humans and animals through contact, or consumption of contaminated food of animal origin, mainly eggs, milk, meat and chicken. Clinical manifestations of Salmonellosis include fever, diarrhea, and abdominal pains within the first 3 days of infection. Some cases may be self-limited while others may require medical care and hospitalization. According to Centers for Disease Control and Prevention (CDC) report, Salmonella causes about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths in the United States every year. Food is the source for about 1 million of these illnesses [2]. Moreover, the economic costs associated with Salmonella illnesses are staggering, ranging from \$4 billion to \$11 billion dollars annually [3]. Antibiotic are used for treatment. However, resistant strains of this bacterium are a handicap to antibio-therapy. Salmonella infections is a worldwide disease present in every country with high prevalence in areas of intensive animal husbandry, especially poultry, cattle, and pig farming [4]. In this study, we are reporting a case of salmonellosis detected in a pig farm where the herd man was the source of contamination. Antibiotic treatment on both animals and human was based on sensitivity tests.

Story of the Case

In 2016, continuous mortality was reported in a pig farm of 50 animals in a locality of the South region of Cameroon. Young piglets were the most affected. Animals were also showing signs of diarrhea and lack of appetite. Fecal samples and blood were collected from diseased pigs following the visit of the mobile epidemio surveillance team from the Laboratoire National Veterinaire (LANAVET) Annex Yaounde. It was also reported that the main herd man in charge of feeding the pigs and cleaning the housing was suffering from signs of salmonellosis. Collected samples were first by PCR for rapid and accurate result. In order to determine the sensitive antibiotics for treatment, antibiogram test was used. Laboratory results confirmed the case of salmonellosis with good sensitivities for Neomycin and Gentamycin. Neomycin was used to treat animals while and the human case was subjected to Gentamycin therapy. From the history, the herd man started feeling signs of salmonellosis two week before being employed in the farm; and no sign of disease was observed nor reported in the farm prior to this arrival. In addition, a week after he started working in the farm, cases of diarrhea with mortalities in piglets were observed in the pig farm. This suggest that the disease may have spread from the herd man to the pigs.

Materials and Methods

A total of 30 samples comprising of 15 anal swaps and 15 blood samples from clinically sick 10 piglets and 5 adults pigs were collected and transported to LANAVET Annex Yaoundé for analysis. Blood and swap samples were first subjected to PCR. After the PCR results, bacteriological analysis of swap samples using isolation and identification were followed by antibiogram.

Molecular Detection of Salmonella sp.

We extracted DNA from blood and swap samples using Qiagen kit's protocol and used PCR to search for a 284 bp fragment of InvA gene of Salmonella as described by Malorny et al. [5] with a slight modification. In brief, a 50ul mix containing 25.5ul of water, 5ul of 10x buffer (Qiagen), 3ul of 25mM Cl2Mg, 4ul of 10mM dNTPs, 4ul of 5mM of each Sal-139 (GTGAAATTATCGCCACGTTCGGGCAA), Sal-141 (TCATCGCACCGTCAAAGGAACC), 0.5ul of 5u/ul Taq Polymerase and 4ul of DNA. The mix was run on a CFX 96 thermocycler (BioRad) with the following cycling conditions: 95°C for 5min followed by 40 cycles of 30 sec at 95°C, 68°C and 72°C each, and a final extension of 5 min at 72°C. PCR products were visualized on UV illumination after electrophoresis in a 1.5% agarose gel.

Isolation and Identification of Salmonella sp.

We cultured, isolated and identified Salmonella as described by Nair et al. [6] with slight modification. Briefly, sample from the transport swab was pre-enriched in peptone water at 37°C for 18 h and then cultured on Rappaport-Vassiliadis broth and Xylose Lysine Desoxycholate (XLD) agar. Characteristic colonies were subjected to passages on the same media before selection for biochemical test using API-20E kit according to the manufacturer's protocol.

Antimicrobial Sensitivity Test

To detect the sensitive antibiotics for the treatment of this case, Salmonella isolates were tested against some antibiotics as described by Kalambhe et al. [7] with slight adjustment. In brief, diluted overnight colonies were spread on Müller-Hinton

agar using sterile swaps. Antibiotic discs (tetracycline (TE:30 μ g), trimethoprim (Tr: 5 μ g), neomycin (N: 30 UI), penicillin (P: 6 μ g /10 IU), gentamicin (GEN: 120 μ g)) were placed accordingly on the agar. The plate was incubated for 24h at 37°C. We used inhibition size of each disk to characterize sensitive and resistant drugs.

Results and Discussions

Both PCR assay and isolation and identification detected Salmonella sp in the samples. All the swap and only two blood samples were positive with PCR, showing approximately 284 bp fragments. Furthermore, Api-20E findings were also identical to Salmonella with 99% confidence. In addition, the antibiogram test indicated that the strain was sensitive for Gentamycin (2.2cm) and Neomycin (2.3cm), but resistant to penicillin and tetracycline. This confirmed the abusive use of antibiotics in animal farming. Among the most used drugs in veterinary medicine, penicillin and tetracycline long acting are on the first line.

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