Albanian j. agric. sci. 2015;14 (2): 192-197 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

New Outbreaks of Salmonellosis in Pig Farms

SULO KOTORRI[1], JONIDA BOCI[2], PELLUMB MUHEDINI[2]

[1]Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania
[2]Institute of Food Safety and Veterinary, Tirana, Albania

Abstract

Salmonellosis is an infection wideley spread in both intensive management and back yard pig farms in Albania. The pathogen agent of this infection is *S. cholerasuisi* var. *Kunzerdof*. This patogen was isolated from untreated piglets with antibacterial drugs during septicemic form of infection from several organs and tissues of aborted fetuses, such as: spleen, lungs, mesenteric lymph nodes, liver, and from jejunum, ileum and stomach content. Biochemical confirmation was conducted by using the commercial kit test API 20E, while biovar typisation was realised using the agglutination test with specific sera of *S. cholerasuis* var. *Kunzendorf*. Despite the level of the serum titer in aborted sows and survival piglets was at low levels, it represents a determinative diagnostic test. Use of antibacterial prophylaxis in feed or drinking water may reduce the incidence of the disease, but does not prevent infection and/or elimination of *S.choleraesuis*. This practice is expensive, increases the antimicrobial resistance, and generally is the less acceptable option for the prevention and control of disease. Using specific preventative vaccine produced with the *S.cholerasuis* var. *Kunzerdof* C-500 strains, showed effectiveness in both farms. Thus today, we can still say that farms are free from *Salmonella* infection.

Keywords: Salmonellosis, *S. cholerasuis*, agglutination test, API 20E, vaccine.

1. Introduction

Salmonella is a genus of rod-shaped, Bacillus. Salmonella species are Gram-negative, flagellated facultatively anaerobic bacilli characterized by O, H, and Vi antigens. There are over 2600 known serovars which current classification considers to be separate species. Salmonella are non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (flagella that are all around the cell body). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes [11; 13].

Salmonella are potent pathogens. They grow at temperatures 7-450C, survive freezing and high temperatures, and stay for weeks, months, or years in suitable organic substrates. An important factor to the success of Salmonella *spp.* as virtual universal patogen is their ability to adapt to a numerous of hosts, many of them serve as resorvoirs. Infections caused by members of the Salmonella, in particular the disease caused by *S. choleraesuis* in pigs produces acute septicaemic or focal infections and/or severe acute diarrhoeic syndrome [9; 12]. Salmonellosis, the disease that may result from Salmonella infection, continues to have a significant economic impact on the national swineherd [8; 9]. S. choleraesuis was one of the most common organisms isolated from cases of swine pneumonia and septicemia. It also was the most prevalent serotype (>90% of isolates) of all Salmonella isolated from diseased swine. In 1991, it was estimated that the disease caused by *S. choleraesuis* cost pork producers in the United States more than $100 million annually due to death losses, medication costs, and poor production efficiency of survivors. Since the mid-1990's, the prevalence of *S. choleraesuis* associated disease has moderated. Currently, serotypes of Salmonella other than *S. choleraesuis* have increased in frequency and presently account for over 50% of the serotypes isolated from diseased swine [1;7; 11]. The disease is spread worldwide. Out of many serotypes of salmonella that exist, the ones that are most likely to cause clinical disease in pigs are *Salmonella choleraesuis*, and *Salmonella typhimurium* and to a lesser extent Salmonella derby. In addition, concerns over Salmonella-contaminated pork products as a human health hazard add to processing and monitoring costs of pork products and may impact overall product demand. All Salmonella *spp.* are considered pathogenic for humans but Salmonella choleraesuis is rarely isolated from pork products [12]. Salmonellosis in swine occurs as either of two general clinical disease entities. Enterocolitis (diarrhea) may be caused by a broad range of Salmonella serotypes, including S. choleraesuis , but

*Corresponding author: Sulo Kotorri; E-mail: sulo.kotorri@yahoo.com
(Accepted for publication June 05, 2015)
***ISSN**: 2218-2020,*

S. typhimurium is more common. Septicemia (affecting multiple organ systems) is due to Salmonella choleraesuis. Salmonella are notoriously diverse. This publication discusses only *S. choleraesuis* as a cause of disease in swine [1; 7; 11; 12]. The acute septicemia form is the most common. Morbidity varies widely, and it could reach up to 50% of heard bred, and mortality ranges from 10-15% of affected animals. As the infection progresses, septicemia will be followed by localization of *S. cholerasuis* to the central nervous system, uterus of pregnant animals, extremities and abdominal cavity, and may cause clinically: meningoencephalitis, abortions, osteitis, and dry gangrene in terminal parts of the body. Also this pathogen is localized in mesenteric lymph nodes, liver, spleen, joints causing hepatitis, splenomegaly, synovitis. Usually, there is extension to regional lymph nodes and, occasionally, generalized septicemia. The most consistent systemic lesion of *S. choleraesuis* infection is interstitial pneumonia and multifocal hepatic necrosis. Pigs may become long-term sub-clinical carriers of *S. choleraesuis*, the organisms surviving in the mesenteric lymph nodes draining the intestine. Many such carriers do not shed the bacteria in faeces unless they are stressed. Pigs may be intermittent or continuous faecal shedders of other serotypes but the carrier state is usually short, weeks or a few months and is self-limiting [10; 13].

2. Material and Methods

This study was conducted in swine breeding farms in Lushnja and Fier regions. In these farms sows, nursing piglets and pigs are bredding. In February 2013 abortions in pregnant sows were observed. In piglets aged over 16 weeks sudden death without premonitory signs was observed, while the other piglets shows pyrexia, rapid development of weakness, depression, inappetence, pneumonia or respiratory distress, cough, the skin of extremities and abdomen (i.e. tail, ears, nose and feet) was slightly red to dark purple (cyanosis). Foul-smelling watery diarrhea which may be blood stained, was a common feature. The level of abortions in pregnant sows ranged from 19 to 28%, while mortality in piglets aged over 16 weeks ranged from 17-25% in the farms included in the study. To isolate the pathogen of the disease samples from piglets abort fetuses as well as dead piglets aged over 16 weeks were taken. For the isolation of Salmonella *spp.* these pathological materials were used:

- suitable material from foetus: spleen and liver
- from piglets aged over 16 weeks, liver, spleen, lung, mesenteric lymph nodes and content from jejunum and ileum.

Each samples was preenriched in buffered peptone water and then added Rappaport-Vassiliadis (RV) and Muller-Kauffman tetrathionate-novobiocine enrichment broth and incubated at 42°C for 24 h. A loopful of the suspension was plated onto Xyloze lysine deoxycholate, agar SS dhe agar MacConkey, agar SS and agar MacConkey plates and incubated at 37°C for 24±3 h [8; 9; 10]. Biochemical confirmation, serotyping of isolated Salmonella spp. strains and serological testing of piglets and aborted sows. Biochemical confirmation was carried out by using API 20E system. Growed colonies on solid media were suspended on 5 ml phisiological solution were pure on wells of API 20E comercial kit. All colonies growed on solid media and have Salmonella *spp.* features and confermed on API 20E test, undergone on serotyping proces by using slide agglutination test according to typospecific sera of S. cholerasuis var. Kunzendorf strain. Sera from each aborted saw and piglets with clinical disease were tested to detect specific *S. cholerasuis* antibody by using microaglutination standard test. Each sera was titreted in range from 1:2 up to 1:256. As antigen we used vaccinal strain of *S. cholerasuis* var. Kunzendorf C-500. Optic density (OD) of S. cholerasuis used in laboratory was judged to be the same with OD of McFarland standard 1. As positiv control sera was used comercial antisera of *S. cholerasuis* (CNEVA, Sophia Antipolis, France, Salmonella O Antiserum Factor 7), while the negative control sera was used piglets foetus sera [10;13].

Notes: Salmonella *spp*. strains that do not produced H2S on XLD agar have dark rose color, while lactose negative strains on XLD agar have yellow color, with or without H2S.

3. Results and Discussion

Isolates obtained from aborted piglets were isolated from the stomach, while isolates obtained from piglets aged 16 weeks were isolated from the liver, spleen, mesenteric lymph nodes, and the content

of jejunum and ileum, following growth on specific before enrichment, enrichment medium and streaking on specific agar: SS agar, XLD and MacConkey agar [10]. *S. cholerasuis* appear as red transparent colonies with black centers on XLD agar. *S. cholerasuis* appear colonies appear as transparent or translucent colorless colonies with black centers on SS Agar, while the medium will turn from yellow to brown. Gram-negative bacilli were identified on microscopic preparations prepared by solid media using Gram stain method [9; 12].

Table 1. Pathological material used to isolate the *Salmonella spp.*

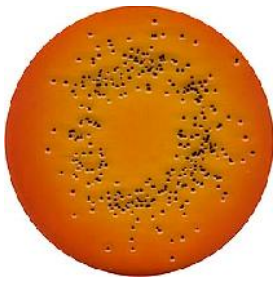
No	Patological Samples	Piglets Foetus	Piglets aged 16 weeks
1	Liver	-	+
2	Spleen	-	+
3	Stomach	+	
4	Lymph nodes	-	+
5	Jejunum	-	+
6	Ileum	-	+

Figure 1. Salmonella *spp* colonies in the XLD agar.

Following incubation, the MacConkey Agar was examined for typical colonial morphology. Non-lactose-fermenting colonies, such as *Salmonella* spp. appear transparent and colorless, with no zone of bile salt precipitation. Biochemical confirmation of the isolated strains gave a positive reaction to the LCD, ODC, H2S GLU, MAN, SOR and RHA. Based on these results epizootic strain isolated from pathological samples belongs to the code 4504510 and was identified as *S. cholerasuis [8*; 9; 10].

The slide agglutination test was done on a glass slide with specific sera of *S. cholerasuis* (antigens O 6.7) and read with the naked eye in front of a light source against a black background. All isolates gave a positive reaction within 30 seconds. Based on these results, the isolated strains were identified as *S. cholerasuis* var. Kunzendorf. Sera from aborted sows and piglets showing clinical signs of disease were tested for the detection of antibodies to *S. cholerasuis* using standard microagglutination test. The antibody *titers* in serum of aborted sows against *S. cholerasuis* ranged from 1: 8 to 1:16, while the antibody *titers in serum* of piglets ranged from 1: 4 to 1: 8 *[11*].

Figure 2. Salmonella spp colonies in the SS agar.



SS agar result the higher selectivity media compared to other specific media used in this study. Isolates of *S. cholerasuis* var. Kunzendorf on SS agar are non-lactose fermenters and therefore developed colonies appear with black centers due to the production H2S. In both pigs breeding farms, piglets aged over 16 weeks developed hyperacute septicaemic form as well as the acute form of the disease, while in sows the abortion was the more pronounced clinical signs. In the acute form of the disease, which was the most frequent, the main clinical symptoms were: anorexia, high fever 40,5-41,60C, cough and respiratory distress. The ears, tail, nose, feet, and abdomen usually become light red to dark purple. Pigs that survive three or four days developed yellow diarrhea containing flakes of fibrin or, less frequently, blood. Postmortem examination revealed purplish discoloration of the extremities and abdomen, swelling and hemorrhage of lymph nodes, and frequently small hemorrhages in many tissues. The lungs were often fluidfilled, did not collapse, had interlobular edema, and usually were diffusely red with small hemorrhages. The spleen usually was swollen and dark blue-black in color. The liver was swollen and blood-filled, and in some cases, contains pinpoint white foci. The inner lining of the stomach was dark red to black. If the disease course had been prolonged or in those pigs with diarrhea, often there were irregular ulcers and fibrin in the large, and sometimes small, intestine. Rarely, there are shallow, oval ulcers throughout the colon [3].

Weaned pigs less than 5 months of age were most often affected. However, market weight and adult pigs occasionally may be affected. Suckling piglets infrequently develop clinical disease due to *S. choleraesuis* . The reason for the resistance of nursing piglets is not known; but is thought to be due to maternal immunity acquired via colostrum and milk.

Disease can occur in suckling pigs in those herds previously naive to S. choleraesuis infection. Abortions also may result from infection of pregnant sows.

Disease due to *S. choleraesuis* consists of acute septicemia (generalized infection of blood and organs) and/or enterocolitis (diarrhea). In both farms, acute septicemia without enterocolitis was by far more common. Salmonella choleraesuis is the only species that commonly results in acute septicemia in pigs. This observation is supported by the work of many different authors who emphasize that *S. choleraesuis* is the only serotype that commonly results in acute septicemia in pigs, while Salmonella typhimurium, and a few other species, usually cause enterocolitis, with septicemia a possible but minor feature [1; 8; 9].

Septicemic *S. choleraesuis* must be differentiated from other acute diseases of swine, such as septicemia due to *Erysipelothrix rhusiopathiae*, *Streptococcus suis*, or Actinobacillus suis, edema disease due to *Escherichia coli*, pleuropneumonia caused by *Actinobacillus pleuropneunoniae*, mulberry heart disease, hog cholera, and more recently, PMWS (post-weaning multisystemic wasting syndrome, thought to be due to porcine circovirus). Diarrhea due to S. choleraesuis must be differentiated from *S. typhimurium*, swine dysentery, porcine proliferative enteritis (ileitis or Lawsonia intracellularis), whipworm (*Trichuris suis*) infestation, and hog cholera (classical swine fever) [1; 3].

Definitive diagnosis was determined when the organism was isolated and identified. In most cases, *S. choleraesuis* was isolated from unmedicated septicemic pigs by directly culturing liver, spleen, intestine, or lymph nodes of aborted fetuses onto blood agar plates and incubating overnight. Isolation from the intestine or feces often is unrewarding even with enrichment techniques. If attempted, enrichment with tetrathionate or Vassiliadis broth is preferred because selenite broth inhibits the growth of *S. choleraesuis*. Biochemical and serological tests are used to provide identification of genus and species. Despite the antibody titers in serum against *S. cholerasuis* of aborted sows and piglets was at low levels using Coagglutination (CoA) test, this technique remains a guiding test to determine the diagnosis. Serology tests are generally not available, lacking both sensitivity and specificity for disease diagnosis. Serology tests (mixed-ELISA) may be used to determine if a herd has had a recent Salmonella infection but are not specific for *S. choleraesuis* [12].

3.1. *Treatment and Prevention*

When treating an outbreak of any disease, the goals are to cure clinically ill pigs and to prevent the spread of the disease to other pigs. In these farms besides using antibacterial agents to reduce the severity of clinical signs of disease, measures were taken for the isolation of infected animals in order to prevent the massive spread of infection. In these farms to prevent the recurrence of infection a modified strain vaccine of S. cholerasuis var. Kunzerdof C-500 was used, acting effectively to control the disease [7] Salmonella spp. are often resistant to many of the antibacterial agents approved for use in swine. Also, *S. choleraesuis* can survive within the host's cells, and few antibacterial drugs are able to enter animal cells. In many outbreaks, less then 10% of the pigs are affected, so the effectiveness of treatment is difficult to evaluate. Despite this, the use of antibiotics for treatment of enteric salmonellosis is supported by some authors [2 ; 5], but the most of them emphasize that the greater efficiency is achieved when antibiotics are used with prophylactic purpose than for therapeutic effects, as both strains *S. cholerasuis* and *S. typhimurium* in vitro usually show resistance to many antibacterial agents [2; 5; 11; 13].

This phenomenon we encountered in both pig farms where the effect of bacterial agents was limited. In contrast with enteric form, antibacterial therapy used to treat septicemic salmonellosis, applied at the beginning of the disease, resulted not only in a decrease of the severity of clinical signs but also in shortening its duration.

In human medicine, antibacterial agents are used to treat septicemic salmonellosis but usually are not used for enterocolitis because antibacterial therapy does not decrease the severity of illness and may actually increase the duration and magnitude of Salmonella shedding in the feces. Experimentally, antibacterial therapy in swine does not appear to increase the severity of the disease nor the duration of Salmonella shedding by clinically affected animals. Antibacterial therapy is viewed by many as beneficial for *Salmonella enterocolitis* and more certainly of benefit for septicemia. Since *S. choleraesuis* usually causes septicemic disease in pigs, antibacterial therapy is warranted in most outbreaks. The use of systemic (able to enter the bloodstream) antibacterials to treat septicemic salmonellosis is widely practiced and is perceived to decrease the severity of the

disease and to increase survival rate. Injectable products often are required for pigs that are clinically affected. Mortality in pigs with purplish discoloration is high, but early treatment combined with separation of animals to small hospital pens will increase survival rate. Antibacterial agents should initially be selected on the basis of susceptibility of the majority of *S. choleraesuis* isolates in the geographic area. Bacterial isolation and antimicrobial sensitivity testing in a particular disease outbreak will allow adjustments in therapy. Anti-inflammatory agents may be of benefit in severely affected pigs [2; 5; 11].

Use of the oral medication (feed or water) beggining early in this outbreak decrease the numbers of new cases by decreasing organism shedding and increasing the dose required to infect healthy penmates. Oral medications are not sufficient to treat disease since affected pigs rarely eat and often do not consume sufficient water for adequate doses of medication to be delivered. The prophylactic use of antibacterial agents in the feed may decrease the incidence of clinical disease but does not prevent infection or eliminate *S. choleraesuis*. This practice is expensive, encourages antibiotic resistance, and is generally regarded as the least desirable option for prevention and control If used prophylactically, feed grade antibacterial additives should be used at treatment rather than at growth promoting levels for short periods of time (pulse doses). To decrease the risk of drug resistance, the same antibacterial drug should not be fed continuously[4; 6].

In the early 1970's, a very effective modified-live, avirulent vaccine (MLV) was introduced by Institute of Veterinary Research to prevent and control *S. choleraesuis* infections. If properly administered, this product control disease well with a single vaccination, is safe, and have duration of immunity that can provide protection until pigs are marketed. There are now at least three such products that can be administered intranasally, intramuscularly, or orally to pigs as young as one week of age. Since these vaccines contain live organisms, the producer must be certain that it is not used while pigs are consuming antimicrobials or water treated with medications or chlorine. The effectiveness of these products are in no small part responsible for the decrease in prevalence of clinical *S. choleraesuis* infections witnessed over the past decade. It is not unusual to see a favorable response (decreased duration and severity of the outbreak) by vaccination of affected groups of pigs. Most producers will then choose to vaccinate the next group of pigs entering the facility to help break the cycle of infection. In those environments or flows of pigs where likelihood of infection or disease is high, vaccine is applied to all pigs produced. In other situations, vaccine can be used intermittently to effectively control disease in the production system. This vaccine provides immunity which lasts for a period of more than 6-7 months. The effectiveness of this vaccine appeared not only in reducing the prevalence of infection caused by *S. choleraesuis* , but also significantly affected in breaking the cycle of infection. In this study, piglets 30 days of aged and sows 3-4 weeks before birth were vaccinated. The vaccine was applied to all pigs in both farms, and today we can say that the farms are free from Salmonella infection [4; 6; 7].

Clinical observations indicate that vaccination reduces but does not always eliminate salmonellosis in the herd. Pork producers should keep in mind that immunity can be overwhelmed by large numbers of organisms in herds with poor sanitation. Concurrent diseases (e.g. pseudorabies, PRRS) at the time of vaccination will also decrease effectiveness. Therefore, management efforts to provide a reduced-stress environment and to reduce fecal shedding and exposure are still important in controlling *S. choleraesuis* in a vaccinated herd.

4. Conclusions

Cause of salmonellosis in swine breeding farms is *S. cholerasuis* var. Kunzerdof. The terrain with higher selectivity resulted SS agar terrain. Biochemical Confirmation is accomplished with commercial kit API 20 E, while sorting at biovari level was conducted with agglutination test with specific type serum *S. cholerasuis* var. Kunzendorf . For specific prophylactic against salmonellosis infection in swine breeding farms was used the lyophilized vaccine with modified strain of *S. cholerasuis* vra. Kunzerdof C – 500.

5. References

1. Adam Moeser. 2005. **Outbreak of salmonellosis in pigs with PMWS.** Vet. Rec 16:92-99.
2. Aserkoff B., Bennett J.V. 2000. **Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae**. N. Engl. J. Med. 281:636-640.
3. Baskerville A., Dow C. 1973. **Pathology of experimental pneumonia in pigs produced by *Salmonella cholerasuis***. J.Comp.Pathol 83:207-215.

4. Cherubin C.E, Neu H.C. et al 1974. **Vaccines and cell-mediated immunity**. Bacteriol Rev. 38:371-402.

5. Finlayson M., Barnum D.A. 1973. **The effect of chlortetracycline feed additive on experimental salmonella infection of swine and antibiotic resistance transfer**. Can. J. Comp. Med 37:139-146.

6. Hanna J. Et al. 1979. **Immunization of pregnant sows with a live *S.cholerasuis* vaccine**. Vet. Microbiolo 3:303-309.

7. Heard T. et al. 1999. **The control and eradication of salmonellosis in closed pigs**. Vet. Rec 82:92-99.

8. Kent J. Schwartz, 2000. ***Salmonella choleraesuis* in Swine**. Originally published as PIH-131.

9. McCaughey W.G. et al. 1986. **Salmonella isolation in pigs**. Vet. Rec.92:191-194.

10. Morse J.W., Hird D.W. 1984. **Bacterial isolated from lymph nodes of California slaughter swine**. Am. J. Vet. Res. 45:1648-1649.

11. Vico JP, Rol I, Garrido V, San Román B, Grilló MJ, Mainar-Jaime RC. 2011. **Salmonellosis in finishing pigs in Spain: prevalence, antimicrobial agent susceptibilities, and risk factor analysis**.

12. Wilcock B.P 1979. **Experimental Klebsiella and Salmonella infection in neonatal swine**. Con.J.comp.Med. Vol.43 200-205.

13. Wilcock B.P., Schwarts K.J. 1995. **Salmonellosis. Bacterial Diseases**.