Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2024 Sept. Vol 6 Issue 3: 156-161

Original Research

Prevalence of Salmonellae infections in sheep and goats and the possibilities of lizards in infection transmission cycle in Maiduguri and environs

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ABSTRACT

This study was conducted to determine the prevalence of Salmonellae infections in sheep, goats and in lizards in Maiduguri and environs. A total of 210 rectal swab samples representing 70 samples each from sheep, goats and lizards were examined according to standard techniques. The results revealed a prevalence of 12, 14 and 12 respectively of positive Salmonellae isolates. Four (4), six (6) and five (5) of these isolates were respectively from male sheep, bucks and male lizards while seven (7), eight (8) and seven (7) of the isolates were from female sheep, goats and female lizards respectively. These accounted for 30.83%, 41.66% and 34.10% respectively for Salmonellae infections in sheep, goats and lizards. The prevalence in males and females were 13.3% and 17.5% for sheep, 15% and 27% for goats and 16.66% and 17.50% for lizards respectively. Antibiotics susceptibility test results showed that the isolates were highly sensitive to ciprofloxacin (32,100%) and ofloxacin (31, 100%). Isolated salmonellae were resistant to Ampicillin and Amoxicillin. Since lizards were found in the vicinity of small ruminants houses, lizards were therefore considered important in the transmission circle of salmonellae to small ruminants. Farmers should be enlightened on the importance of using lizard proof materials in the construction of small ruminants sheds. Pet animals such as cats should be adapted for frequent visits to farm premises where sheep and goats are kept to scare lizards away from the area. Strict hygienic measures should be part of public enlightenment campaigns to minimize the spread of salmonellosis generally in the environment.

Keywords: Goats, sheep, lizards, salmonellae, transmission

INTRODUCTION

Salmonella species are members of family enterobacteriaceae. They are gram negative facultative anaerobic rods and are motile with the means of peritrichous flagella. Salmonella species are classified into serovars (serotypes) based on lipopolysaccharide (0), flagella protein (H), and sometimes the capsular (VI) antigens. There are more than 2,500 known serovars. Within the serovar, there may be strains that differ in virulence (Rodriguez-Calleja *et al*, 2006). Salmonellae have been reported to occur naturally in contaminated environment and thus have attracted the attention of bacterial geneticists (Acha and Szyfres, 2003).

Salmonellosis is an infection of the digestive tract caused by the bacterium, *Salmonella enterica*. *Salmonellae* are Gram negative bacteria which belong to the family enterobacteriaceae and genus *Salmonella* (Yan *et al*, 2003). They are small facultative anaerobic, straight rods, 0.7 x 1.5-5µm in size (Holt *et al*., 2002). There are over 2,200 reported serotypes of *Salmonella*, yet fewer than 2% of these accounted for approximately 80% of the disease reported in livestock, poultry and humans (House and Smith 2003). The habitat of the genus *Salmonellae* seemed to be limited to the digestive tract of humans and animals. Thus the presence of *Salmonella* in other habitats (water, food, and natural environment) is explained by faecal contamination. However, *Salmonella* is not a normal flora of mammals' intestinal tract (Ashworth, 2006). Some serovars (serotypes) have a habitat limited to a host species, such as humans (serovars: *typhi, paratyphi* A), sheep (serovars: *abortus ovis*), fowl (Serovars: *gallinarum, pullorum)* or cattle (serovar: *dublin*). Out of the 2% of salmonellae responsible for the 80% of the disease in man and animal, 95% is associated with sero-groups B, C, D and E. *Salmonella enterica* is an important cause of food borne disease with an estimated 1.4 million illnesses and 5000 deaths attributed to Salmonellosis in the United States. Salmonella infection has

http://doi.org/10.54328/covm.josvas.2024.175

a wide range of prevalence in different species of animals (Angulo *et al.*, 1998; Baumer *et al*, 2000).

The prevalence of salmonella among sheep and goats vary considerably between serotypes, herds and geographic regions. Large outbreak of *Salmonella abortus ovis* among sheep has been reported that infections with these serotypes have contributed to lambing loses of up to 70% between 2003 and 2007 (Fish *et al*., 2008). *Salmonella diarizonae* is the causative agent of winter dysentery, a disease of sheep that is also associated with abortion and still birth. Serotypes diarizonae represents another common sheep adapted serotypes. The prevalence of serotype diarizonae in the Norwegian sheep herds has been estimated approximately to be 12% prevalence within herd and in the range of 0-45%, even though the samples were collected from abattoir and as such stress might have contributed to the high prevalence (Fuller *et al*., 2008). Late term abortion, mortality in ewes, and high kids mortality can lead to extensive economic loses in sheep and goats operations making Salmonella abortion one of the economically important disease of small ruminants (Meerbug, 2006). Abortion due to infection with serotype such as typhimurium or dublin have been reported, but abortion is mostly and frequently caused by *Salmonella arbortus ovis*, ovine adapted serotypes that also occasionally infects goats, and usually abortion generally occurs in the last weeks before parturition.

Salmonellae occur naturally in gastrointestinal tract of many reptiles commonly shed by these animals. Across the world, a large number of serotypes of salmonellae have been isolated from feral and captive reptiles as well as their eggs. Clinical disease including septicemia, osteomyelitis, salpingitis, nephritis, dermatitis and abscess, seemed to be associated with Salmonellae infections in lizards but overwhelming majority of infections in reptiles is rear and undoubtedly asymptomatic (Silbergeld *et al*., 1984).

Clinical Salmonellosis in Lizard's is rare, appears to be associated with underlying disease or other stressors, and a causal relationship between Salmonella infections and disease is generally difficult to establish conclusively (Bemis *et al*., 2007). Whether Salmonella infection causes diarrhoea in Lizard's is still subject to debate, and might depend on variety of factors including host species and ambient temperature during infection (Pasmans *et al*., 2002).

A number of Salmonellae serotypes have been found associated with reptiles and or amphibians including, the *S. enterica* sub sp *enterica serovars chameleon, java, manna, poona, stanley* and *typhimurium*, among others. *S. bongori, S enterica* subsp *salamae, S. enterica* subsp *arizonae, S enierica* subsp *diarizonae, S. enterica* subsp *houtenae, S. enterica* subs *indica*, are usually found in poikilotherms including reptiles (lizards) and in the environment (Acha and Szyfires 2003).

Prevalence estimates in free ranging reptiles vary widely and a few studies reported the absence of Salmonella in their study populations (Gartrell *et al*., 2007). It has been estimated that as many as 90% of all captive Lizards carry Salmonella including a large number of reptile-associated as well as broad host- range serotypes (Woodward *et al*., 1997).

The efficacy of antimicrobial treatments was studied extensively by array of researchers and in some instances it was combined with treatment regimens that will help reduce the carriage of salmonellae pathoens by reptiles. Some laboratory investigations carried out in this respect were positive (Silbeling et al., 1984). In experimental studies, constraints such as routine treatment of reptiles , eggs, or pond water on commercial farm were experienced and were established to be as a result of farming conditions, intermittent Salmonella shedding, trans-ovarian infection, coprophagy and environmental reservoir. The treatments appear were established as having heightened risk of antibiotics resistance as reported by Mitchell et al. (2007). Infection with Salmonella has been detected to the level of isolation of the pathgoen from commercially raised Lizards, Turtles, Crocodile, Alligators and Iguanas and substantial contamination was recorded from the meat or the farm environment, with prevalence estimates for farm lizards, turtles as high as 40%. The stress associated with transportation further aggravates Salmonella shedding, especially among younger Lizards and Turtles.

The study aimed to detect the presence of Salmonellae infections from sheep, goats and lizards in Maiduguri and environs and also evaluate antibiotic susceptibility testing to establish better drug(s) of choice for treatment of Salmonellosis in these species was also one of the objectives of the study.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 210 rectal swabs were randomly collected from sheep, goats and from lizards comprising of 30 each from male goats, sheep and lizards and 40 each from female goat, sheep and lizards in small holder ruminant farms in Maiduguri and environs. Large animal clinic of Veterinary Teaching Hospital and Department of Animal Science farm University of Maiduguri were some of the locations where samples were collected. The swabs were inserted into the rectum at angle of 45^0 and samples were collected by scrubbing gently in the anal lumen. Ethical approval for the study was obtained from the ethical committee, University of |Maiduguri (No: FVM/ETHICS/1257/2024/UNIMAID).

MEDIA PREPARATION

Different types of culture media were prepared in the laboratory of Department of Veterinary Medicine for the isolation of Salmonellae organisms from rectal swab samples

collected. These included: Selenite F. broth, Brilliant green Agar, nutrients broth and nutrient Agar. These media were prepared according to the manufacturer's instructions in sterile petri dishes for the isolation of pure colonies of Salmonellae. Sub-culturing was done to obtain pure colonies of Salmonellae after the first inoculation.

SELENITE F BROTH

This media was used as an enrichment media before inoculation of the sample onto a primary media. The constitution factor is 19g/L if water. Appropriate volume is prepared in a clean glass flask and sterilized under a pressure of 15mmHg, a temperature of 121 °C for 30 minutes. This is allowed to cool to a temperature of 40 - 45°C. It was then dispensed carefully into sterilized Bijou sample bottle.

MAC CONKEY AGAR

This was used as the primary media. It was constituted at 54g/L of water in a clean glass flask and sterilized under a pressure of 15mmHg, a temperature of 121°C for 30 minutes, allowed to cool to a temperature of 55 - 60°C and poured gently into sterile Petri dishes (20ml/Petri dish). The poured media was then allowed to set and was dried in an incubator at a temperature of 40 - 42°C.

BRILLIANT GREEN AGAR

This media was used as a selective media for salmonella. It was prepared at 58g/L of water in a clean glass flask, sterilized in an autoclave under a pressure of 15mmHg, a temperature of 121 °C for 30 minutes, allowed to cool to a temperature of 55 - 60°C and poured carefully into sterile Petri dishes (20ml/Petri dish). The poured media was then dried in incubator at a temperature of 40 - 42°C.

NUTRIENT AGAR

This media was used as an enrichment media for the positive isolates before culturing for antibiotic sensitivity test. It was prepared at 15g/L of water in a clean glass flask which was then sterilized in an autoclave at a pressure of 15mmHg, a temperature of 121°C for 30 minutes and allowed to cool to a temperature of 40-42°C and then gently dispensed into sterilized Bijou bottles.

PEPTONE WATER

This media was used for antibiotic sensitivity test. It was prepared at 28g/L of water in a clean glass flask, sterilized in an autoclave under a pressure of 15mmHg, a temperature of 121°C for 30 minutes, allowed to cool to a temperature of 55°C - 60°C and gently poured onto sterile Petri dishes. This was allowed to set and was dried in an incubator at a temperature of 40°C - 42°C.

SAMPLE PROCESSING

Samples collected were taken to the Veterinary Medicine Laboratory, University of Maiduguri for the isolation of Salmonellae. The samples were first inoculated into selenite F broth and incubated at a temperature of 37°C for 24 hours. A loopful of the cultured sample was then inoculated onto MacConkey agar and then incubated at 37°C for another 24 hours. Distinct colonies that showed characteristic lactose fermentation were suspected for Salmonella or Shigella. The suspected colonies were inoculated on brilliant green agar; a selective media for Salmonella for differentiation. Positive isolates were then sub-cultured in peptone water before testing for antibiotic sensitivity.

MOTILITY TEST

This test was used to differentiate Salmonella organisms from Shigella species which were comparatively non-motile. The principle of the test demonstrated the ability of viable Salmonellae to move freely in a liquid medium and no-motility in the case of Shigella species. Using a wire loop, a colony was picked from the growth on blood agar and was used to prepare a saline solution and a drop of the solution was applied on glass microscope slide and covered with a cover slip. The solution was surrounded with plasticine which covered the microscope slide. The preparation was then inverted so that the coverslip comes on top. This was observed under microscope at x 10 objective lens and later at × 400 objective lens. Distinct movement of bacteria amidst Brownian motion indicated that the isolated organism was motile.

ANTIBIOTICS SENSITIVITY TEST

The antibiotics disc was commercially purchased for this purpose. To test for antibiotic sensitivity of the positive isolates, one milliliter of the sample sub-cultured in the peptone water was aspirated using a pipette and instilled on the surface of the prepared nutrient agar. The sensitivity disc was then placed at the center of the nutrient agar and incubated at 37°C for 24 hours.

PROCEDURE FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

The prepared nutrient agar was poured into sterilized petri dishes. Peptone water was also prepared into universal bottles and inoculated with suspected Salmonellae organisms recovered from nutrients agar slant and allowed to stand for 48hrs at 37°C. About 2-3 drops of the incubated peptone water was poured on the nutrients agar in the petri dishes and the antibiotic impregnated disc was placed on it. This was incubated for 48hrs to observe for zone(s) of inhibition of Salmonellae growth. If the organism was susceptible there will be zone of inhibition around area of growth and if not susceptible there will be no zone of inhibition on the antibiotics disc.

Table 1: Antibiotics on Disc with Codes and Concentration employed for Sensitivity Testing

Antibiotics	Code	Concentrations
Augmentin	AUG	30ug
Ceftriazone	CRD	30ug
Nitrofiiratoin	NTT	200ug
Gentamycin	GEN	10ug
Cotrimozazole	COT	25ug
Ofloxacin	OFL	5ug
Amoxicillin	AMX	25ug
Ciprofloxacin	CPX	10ug
Tetracycline	TET	30ug
Pefloxacin	PFX	5ug

RESULTS

Examination of a total of 210 rectal swab samples representing 70 samples each from sheep, goats and lizards revealed the prevalence of 12, 14 and 12 respectively of positive Salmonellae isolates. Four (4), six (6) and five (5) of these isolates were respectively from male sheep, bucks and male lizards while seven (7), eight (8) and seven (7) of the isolates were from female sheep, goats and female lizards respectively. These accounted for 30.83%, 41.66% and 34.10% respectively for salmonellae infections in sheep, goats and lizards with the breakdown of prevalence in males and females as13.3% and 17.5% for sheep, 15% and 27% for goats and 16.66% and 17.50% for lizards respectively as shown in Tables I, II and III.

Antibiotics susceptibility test results showed that the isolates were highly sensitive to ciprofloxacin - 32(100%) and ofloxacin - 31 (100%). Salmonellae isolated were resistant to Ampicillin and Amoxicillin. These were determined through observations of measurements of zones of inhibition due to Salmonellae growth on the petri dishes around various antibiotics. There were no zones of inhibition around antibiotics to which salmonellae were not susceptible.

Table II: Prevalence of Salmonellae Infection in Sheep in Maiduguri and Environs

Sex of Sheep Examined	Number of Samples Examined	Number of Positive Isolates	Percentage (%)
Male	30.0	4.0	13.33
Female	40.0	7.0	17.50
Total	70.0	11.0	30.83

Table III: Prevalence of Salmonellae Infections in Goats in Maiduguri and Environs

Sex of Goats Examined	Number of Samples Examined	Number of Positive Isolates	Percentage (%)
Male	30.0	6.0	15.00
Female	40.0	8.0	26.66
Total	70.0	14.0	41.66

Table IV: Prevalence of Salmonellae Infections in Lizards in Maiduguri and Environs

Sex of Lizards Examined	Number of Samples Examined	Number of Positive Isolates	Percentage (%)
Male	30.0	5.0	16.66
Female	40.0	7.0	17.50
Total	70.0	12.0	34.10

DISCUSSIONS

The study to determine the prevalence of Salmonellae infections in small ruminants, sheep and goats and lizard and the possible role of lizards in the transmission circle in Maiduguri and Environs revealed the prevalence of 11 (30.83%) and 14 (41.66%) cases of salmonellae infections in sheep and goats respectively. Similarly 12 (34.10%) cases were representing the prevalence of Salmonellae infections in Lizards inhabiting the premises where the small ruminants were intensively kept and obviously contributed to the transmission of salmonellae to sheep and goats. These findings corroborates with assertions of Fisher *et al.* (2008) who reported that prevalence of salmonellae among small ruminants vary considerably between serotypes, speciesherds and geographic regions. Hjartardottir *et al*. (2002) reported a high prevalence of *Salmonella typhimurium* infections in slaughter houses in different countries with prevalence estimated generally in the range of 17-60% and a considerably low prevalence however recorded among slaughtered sheep and goats in India and Ethiopia.

Although, *Salmonella di-arizonae* causes winter dysentery in sheep and goats but was not isolated in the present study in both small ruminants and the lizards but the presence of this species may not be entirely ruled out especially in sheep and goats. This was supported by Fuller *et al*. (2008) who reported the prevalence of *salmonella serotype di-arizonae* in Norwegian sheep herds with an approximate estimate of 12% within a herd and the prevalence ranged from 0-45%, even though screened samples were collected at an abattoir

where increased stress may have contributed to the high observed prevalence rate in this case.

A report by Bemis *et al*. (2007) highlighted the importance of captive lizards in the transmission of Salmonellae infections as the reptile commonly shed large number of serotypes in faeces and eggs. Gartrell *et al*. (2007) further argumented on these claims that in series of researches conducted to establish the presence of Salmonellae infections in lizard populations, very few of the findings indicated the absence of Salmonellae in lizards in the study populations. Furthermore, Woodward *et al*. (1997) also estimated that as many as 90% of the entire captive lizard's carry Salmonella infections in a separate studies conducted to establish the presence of Salmonellae infections in the species of reptiles. These therefore supported the findings of the present investigations were positive Salmonellae were isolated in lizards caught and sampled in premises where sheep and goats were kept and presumably contributed to the transmission of this pathogens to small ruminants.

Antibiotic susceptibility testing of detected salmonellae isolates as conducted in the present study have shown that the isolates were highly sensitive to ciprofloxacin and ofloxacin compared to other tested antibiotics. This was supported by the findings of Mitchell *et al*. (2007) who reported the efficacy of some antimicrobial treatment on Salmonellae infections and stressed the importance of combining chemical treatments with physical treatments in reducing reptiles' Salmonellae carriage in small ruminants. Furthermore, as farming conditions, inter-current salmonella shedding, tran-ovarian infections, coprophagy and environmental reservoirs appeared to be associated with a heightened risk of antibiotic resistance these conditions might be some of the reasons for recording resistance in Ampicillin and Amoxicillin in the present study but which were earlier established as effective antibiotics in the treatment of salmonellae infections in small ruminants in other studies.

CONCLUSIONS

Based on the outcome of the present study, it was concluded that Salmonellae infections are common in sheep and goats. Salmonella species was isolated in sheep, goats and lizards in the same environment in the present study lizards were possibly involved in the transmission circle of salmonellae to small ruminants. Antibiotic susceptibility test results conducted have shown that the isolates were highly sensitive to Ciprofloxacin and Ofloxacin compared to other tested antibiotics. The isolates were also resistant to Ampicillin and Amoxicillin in the present study but which were earlier established as effective in the treatment of salmonellae infections in small ruminants in other studies. Farmers should be enlightened on the importance of using lizard

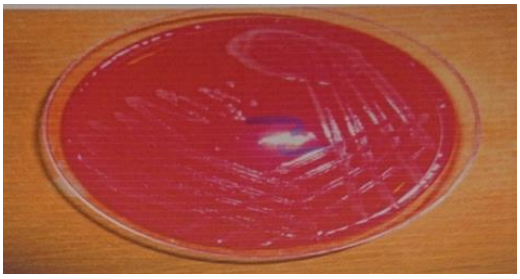

Plate 1: Positive Salmonellae Growth on Blood Agar Plate

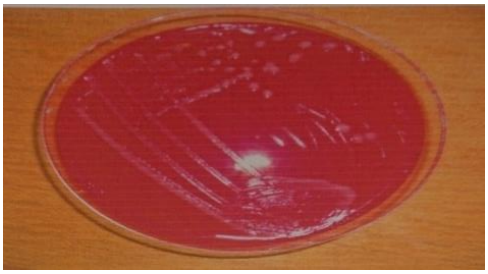

Plate II: Positive Salmonellae Growth on Blood Agar

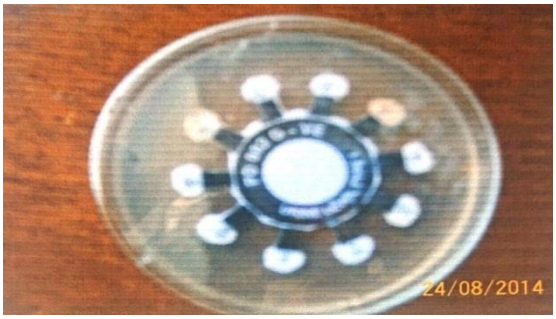

Plate III: Antibiotic Sensitivity Test Disc Showing Zones of inhibition with Salmonellae species

proof materials in the construction of small ruminants sheds. Pet animals such as cats should be adapted for frequent visits to farm premises where sheep and goats are kept to scare lizards away from the area. Strict hygienic measures should be part of public enlightenment campaigns to minimize the spread of salmonellosis generally in the environment.

ACKNOWLEDMENT

The cooperation of small holder ruminant farms and that of Veterinary Teaching Hospital as well as the Animal Science farm of the University of Maiduguri was highly appreciated for given the permission to collect samples for undertaking the study. The cooperation of Veterinarians, Para-vets and all other persons that contributed in making the work successful was equally highly appreciated.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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