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Presence, distribution, serotypes and antimicrobial resistance profiles of *Salmonella* among pigs, chickens and goats in South Africa

M.A. Mathole [a], F.C. Muchadeyi [b], K. Mdladla [b], D.P. Malatji [b], E.F. Dzomba [c], E. Madoroba [a, d, *]

[a] Bacteriology Division, Agricultural Research Council-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110, South Africa
[b] Agricultural Research Council- Biotechnology Platform, Private Bag X05, Onderstepoort, 0110, South Africa
[c] Discipline of Genetics, School of Life Sciences, University of KwaZulu Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209, South Africa
[d] College of Agriculture and Environmental Sciences, University of South Africa, Florida Campus, Christiaan De Wet/ Pioneer Dr. P.O. Box X6, Florida, 1710, South Africa

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ABSTRACT

Salmonellosis is an infectious zoonotic disease of socio-economic importance worldwide. Food animals with subclinical infection as well as farm effluents are usually the sources of contaminated meat, eggs and milk, which cause diarrhoea and systemic infections in humans. The indiscriminate use of antibiotics to curb salmonellosis in both animals and humans has contributed to the emergence and spread of drug-resistant bacteria among both pathogenic and commensal organisms. The aim of the study was therefore to determine the presence, serovar distribution and antimicrobial resistance profiles of *Salmonella* isolated from domestic livestock species in South Africa. For this purpose,1069 rectal and cloacal swabs were collected from pigs (n = 322), chickens (n = 286) and goats (n = 461) from smallholder farms in Limpopo, Eastern Cape, Northern Cape, North West and KwaZulu Natal provinces of South Africa. The frequency of occurrence of *Salmonella* per animal species was highest in pigs (5.90%; n = 19), followed by chickens (3.15%; n = 9) and goats had the lowest proportion of 0.43% (n = 2). Nine *Salmonella* serovars were obtained including S. Techimani, a serovar that was not previously observed in South African animals. Six isolates were assigned to *Salmonella* II. Some of the *Salmonella* were untypable (n = 6). All *Salmonella* isolates were sensitive to cefotaxime, enrofloxacin, florphenicol and polymyxin B. Most of the *Salmonella* isolates were resistant to at least one antimicrobial (n = 20; 66.7%) and resistance was predominant towards trimethoprim (n = 11; 36.7%), followed by ampicillin (n = 5; 16.7%), oxytetracycline (n = 3; 10%), and kanamycin (n = 1; 3.3%). The results illustrate the presence of diverse and rare *Salmonella* serovars that were not previously isolated from animals in South Africa. The pattern of development of antibiotic resistance should be monitored and followed-up. The occurrence of elevated trimethoprim resistant *Salmonella* in South African food animals could lead to the emergence and distribution of drug resistant salmonellosis in human beings.

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1. Introduction

In South Africa, poultry is produced by large commercial farmers, small scale farmers as well as households for eggs and/or meat. Poultry is one of the cheapest sources of meat. A census that was undertaken in 2014 indicated that there were approximately 140 million chickens at any one point in South Africa (South Africa Poultry Association (SAPA, 2014). The majority of chickens were in North West province (21.7%), followed by Western Cape province (20.5%), Mpumalanga (17.0%), KwaZulu Natal (13.6%) and Gauteng province (10.6%) (SAPA, 2014). Goats are found throughout the country and are a source of meat and milk. In 2004, the South African goat population was estimated to be 6.58 million (National Agricultural Marketing Council, 2005). In 2010, the majority of goats were found in the Eastern Cape province (37%) and Limpopo provinces (20%), followed by KwaZulu Natal (13%), North West (11%), Northern Cape (8%), Western Cape and Free State (4%),

* Corresponding author. Bacteriology Division, Agricultural Research Council-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110, South Africa.
E-mail address: MadorobaE@arc.agric.za (E. Madoroba).

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Mpumalanga (2%), and Gauteng province (1%) (Department of Agriculture, Forestry and Fisheries, 2011a). Between 2010 and 2011, the pig population in South Africa was estimated to be 1,584 million (DAFF, 2012), with Limpopo and North West provinces being the largest producers and production has been increasing from 2000 to 2009 (DAFF, 2011b).

Households and smallholder farms in the rural communities keep livestock species under extensive low input farming systems characterised by poor housing, low quality scavenging feed sources and limited veterinary interventions. Livestock species are kept as mixed flocks with minimum biosecurity. The low input production system and limited biosecurity measures expose the different livestock species to various pathogens. These livestock are raised predominantly for food security reasons and they provide households with cheap and readily available source of meat, eggs and milk. This is important from a socio-economic standpoint, but livestock particularly those raised under low input biosecurity systems, may pose a health risk to humans. It is therefore important for the veterinary profession to offer solutions to these systems.

Salmonella serovars are some of the most important causes of food-borne diseases worldwide. Salmonellosis is more likely to be related to animal food products where they act as vehicles for transmission (Mürmann, dos Santos, & Cardoso, 2009). Alcaine et al. (2006) indicated that the *Salmonella* serotypes isolated from farms are linked to the *Salmonella* spp causing diseases in humans. A study in Spain revealed that 40.9% of pig herds were infected with *Salmonella* spp (Arguello, Sørensen, Carvajal, Baggesen, & Rubio, 2013). In South Africa, 19% *Salmonella* spp prevalence was observed in poultry (Van Nierop et al., 2005).

Salmonella infections are related to management issues and their control depends on controlling the source of contamination and transmission. The poultry and pig industries are faced with financial constraints due to these pathogens, and farmers have resorted to the use of antimicrobial agents for treatment, control and prevention. In addition, farmers use antimicrobial agents for production purposes as growth enhancers. These growth promoters are fed to the livestock or poultry to improve their intestinal composition (Hur, Jawale, & Lee, 2011). This action may result in antimicrobial resistance, which is a significant public health threat.

The aim of this study was to determine the presence and distribution, serotypes and antimicrobial resistance profiles of *Salmonella* in domestic livestock species of South Africa. The study targeted chicken, goats and pigs that are kept by smallholder and rural households under low input mixed-livestock farming systems.

2. Materials and methods

2.1. Sample collection

The samples were collected from smallholder farms in Limpopo, Kwa-Zulu Natal, North West, Eastern Cape and Northern Cape provinces of South Africa. The samples were collected from April 2013 to September 2014 and they were analysed within 48 h. One thousand and sixty nine samples (cloacal/rectal swabs) were collected from free-range apparently healthy pigs (n = 322), goats (n = 461) and chickens (n = 286). The samples were placed in Amies transport media and transported to the Bacteriology section of Agricultural Research Council-Onderstepoort Veterinary Institute.

2.2. Microbiological analysis

2.2.1. Bacterial isolation

Each sample was analysed according to ISO 6579, 2002. *S.* Typhimurium ATCC 14028 and *Escherichia coli* 25922 were included as positive and negative controls respectively.

2.2.2. Biochemical tests

All presumptive *Salmonella* isolates were subjected to a battery of biochemical tests according to ISO 6579, 2002. Isolates showing a combination of typical *Salmonella* biochemical reactions were cultured on BTA and incubated at 37°±1 °C for 24 h, followed by serotyping.

2.2.3. Serotyping

Salmonella spp serotyping was done using slide agglutination as prescribed in the White-Kauffmann-Le Minor scheme (Grimont & Weill, 2007; Popoff & Le Minor, 1997). *Salmonella* spp serotyping was undertaken to identify surface antigens (Lipopolysaccharides, O-antigens) and flagella antigens (H-antigens). Each isolate was tested for autoagglutination prior to serotyping. *Salmonella* suspensions that agglutinated on their own without addition of antisera were considered autoagglunating or 'rough cultures' and these were not further serotyped.

For O-typing, loopfuls of saline were separately placed on clean glass slides, followed by mixing with *Salmonella* spp (grown on nutrient agar) until a smooth opaque suspension was formed. Drops of polyvalent O antisera were added to the bacterial suspensions (antigen), followed by mixing for approximately 2 min. Bacterial suspensions that remained homogenous were considered negative, and clumping indicated positive reactions. *Salmonella* isolates that reacted with polyvalent O antisera were further typed with individual monovalent antisera and all reactions were noted.

For H-typing, the *Salmonella* spp colonies were subcultured from nutrient agar and each isolate was separately inoculated on one spot at the centre of Swarm agar, followed by overnight incubation at 37°±1 °C. The bacterial cultures from the edge of the Swarm agar were suspended in saline and mixed with H-antisera pools as described for O-typing. The interpretation of negative and positive (1 Phase) results was similar to that of O-typing. For H-positive isolates, phase inversion was done prior to detection of 2 H-antigen Phase.

The results of both O and H-typing were combined in order to determine the *Salmonella* serovar using the White-Kauffmann-Le Minor scheme (Grimont & Weill, 2007; Popoff & Le Minor, 1997).

2.2.4. Antimicrobial susceptibility testing

All 30 *Salmonella* spp isolates (Fig. 1) were subjected to antimicrobial susceptibility tests. The colonies were inoculated in nutrient broth and turbidity of the suspension was adjusted to 0.5 McFarland standard. A sterile swab was immersed in the nutrient culture broth and aseptically streaked on Mueller Hinton agar in three different directions to obtain confluent growth. Antibiotic disks (ampicillin (10 μg), cefotaxime (30 μg), enrofloxacin (5 μg), florphenicol (30 μg), kanamycin (30 μg), oxytetracycline (30 μg), polymyxin B (300 μg) and trimethoprim (5 μg)] were dispensed onto the Mueller Hinton agar and incubated at 37 °C for 24 h. The plates were examined for zones of inhibition, which were measured in mm and classified as resistant (R), sensitive (S) or intermediate (I) according to Clinical and Laboratory Standards Institute (CLSI, 2014) or the manufacturer.

3. Results and discussion

3.1. Presence and distribution of Salmonella

The presence and distribution of *Salmonella* in goats, pigs and chickens is summarized in Fig. 2. Overall, thirty (2.81%) of 1069 isolates across species were positive for *Salmonella*. Of these

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