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

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%),

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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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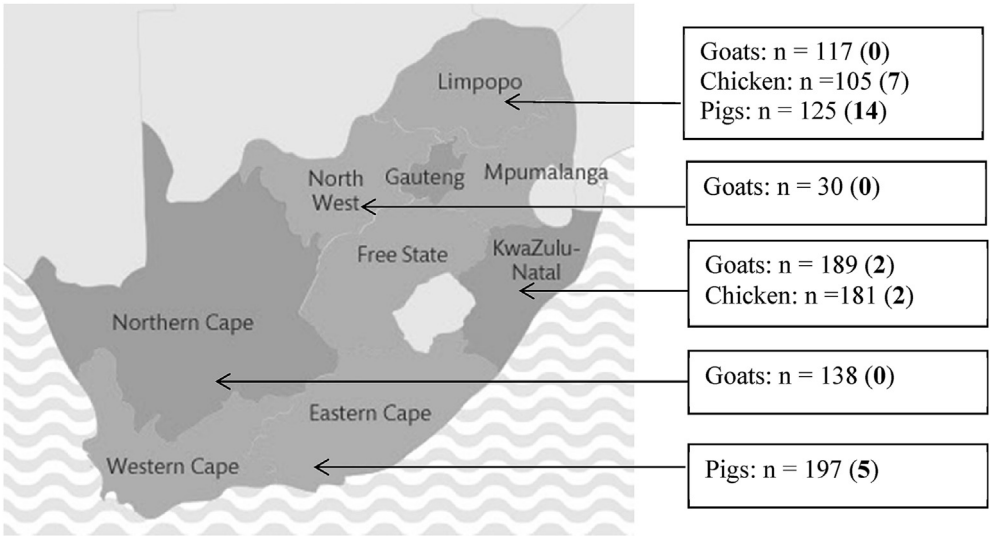

Fig. 1. Map of South Africa showing the 5 provinces were samples from goats, chicken and pigs were obtained. The numbers in brackets indicates *Salmonella* positive samples. Source of map: http://www.southafrica.info/about/geography/provinces.htm#.VyFKSGBJkmw. Accessed on 20 April 2016.

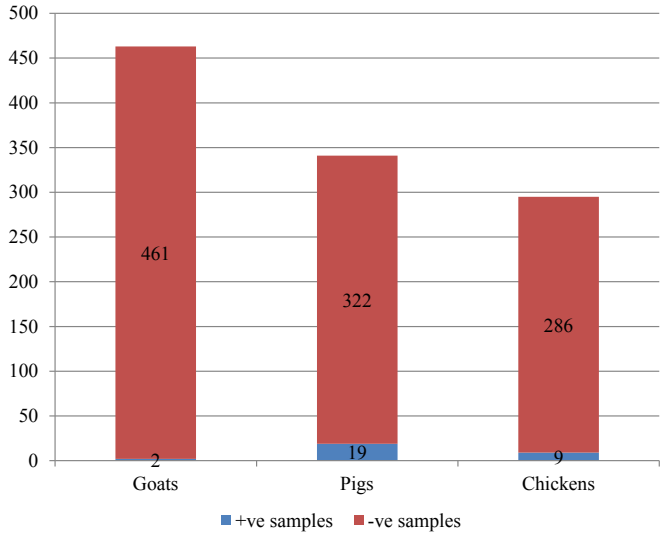

Fig. 2. Presence and distribution of *Salmonella* spp isolated from goats, pigs and chickens. The number of *Salmonella* spp positive isolates is shown in the blue-shaded area of the bar graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

positive isolates, the frequency of occurrence of *Salmonella* spp according to animal species was highest in pigs (5.90%; n = 19), followed by chickens (3.15%; n = 9) and goats had the lowest presence of 0.43% (n = 2).

Despite the low proportion of *Salmonella* from healthy goats in this study, these bacteria have potential of causing environmental contamination, which poses a risk to human beings (Edrington et al., 2009). On the contrary, the prevalence of *Salmonella* in goats was higher in other studies. For instance, in a study that was aimed at determining the prevalence of *Salmonella* in the faeces of cows and goats in the Eastern Cape province of South Africa, Igbinosa (2015) isolated *Salmonella* from 45% (n = 68) and 55% (n = 82) of goat and cattle faeces respectively.

The findings of our study highlights the need for designing and implementing on-the-farm solutions that could be applied by smallholder farmers to circumvent the proliferation of *Salmonella* spp and antibiotic resistance among food production animals. The control of *Salmonella* spp at the farm level is usually complicated, hence a strategy that involves diverse approaches is recommended (Forshell & Wierup, 2006). One of strategies would be to establish the point of infection at the farm level through longitudinal studies from the stage of weaning to animal slaughter (Ball, Magowan, Taylor, Bagdonaite, & Madden, 2011). In addition, administration of comprehensive questionnaires to small holder farmers will assist with identification of *Salmonella* prevalence drivers on the farm. Questionnaire administration will aid the development of statistical models that can be used to identify factors that influence *Salmonella* prevalence (Ball et al., 2011).

3.2. Serotypes

Of the 30 *Salmonella* isolates, 9 serovars were identified (Table 1). A rare serovar, S. Techimani was identified in 9 pigs from Limpopo and Eastern Cape provinces and this serovar was not previously identified in South African pigs. Six of the *Salmonella* isolates were untypeable and could not be further analysed to serovar level. The 24 typeable serovars were assigned to *S.* Chester (n = 1), *S.* Cardoner (n = 1), *S.* Sambrae (n = 1), *S.* Typhimurium (n = 1), *S.* Schwarzengrund (n = 2), *S.* Aarhus (n = 1), S. Pomona (n = 1), *S.* Senftenberg (n = 1) and *S.* Techimani (n = 9) respectively. The six remaining isolates were classified as *Salmonella* II (n = 6).

Table 1 summarises the diversity of *Salmonella* serovars among pigs, goats and chickens in this study. *S.* Techimani was predominant in pigs, whereas *Salmonella* II occurred more frequently in chicken. Five out of the 6 untypeable *Salmonella* were isolated from pigs.

The results illustrate the presence of diverse *Salmonella* serovars among pigs, chickens and goats raised by smallholder farmers in South Africa. These *Salmonella* serovars could pose a risk to smallholder farmers through animal handling or environmental contamination, which usually complicates pathogen control. The predominance of *S.* Techimani among the 30 isolates from this study was unexpected, particularly because this serovar was not identified in a retrospective study conducted over a 11 year period in South Africa, even among the isolates from pigs (Kidanemariam, Engelbrecht, & Picard, 2010). *S.* Techimani was isolated from cattle in a study that was conducted in Ghana in 1961 (Guinee,

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Table 1
Distribution of *Salmonella* serotypes isolated from goats, pigs and chickens.

Geographical origin	Animal species	Number of animals (*Salmonella* positive)	*Salmonella* serovar (n)
KwaZulu Natal	Goats	189 (2)	*S.* Chester (1); *S.* Typhimurium (1)
	Chicken	181 (2)	*S.* Cardoner; (1); Untypeable (1)
Limpopo	Goats	117 (0)	NA
	Chicken	105 (7)	*S.* Sambre (1); *S.* Schwarzengrund (1); *Salmonella* II (5);
	Pigs	125 (14)	*S.* Schwarzengrund (1); *Salmonella* II (1); Untypeable (3); *S.* Aarhus (1); *S.* Pomona (1); *S.* Techimani (7)
Eastern Cape	Pigs	197 (5)	Untypeable (2); *S.* Senftenberg (1); *S.* Techimani (2)
Northern Cape	Goats	138 (0)	NA
North West	Goats	30 (0)	NA

Kampelmacher, & Willems, 1961). Even so, as there is little information available with regards to this serotype; thus it would be paramount to extend the surveillance to more regions in South Africa in order to establish not only the epidemiology of *S.* Techimani, but also its human health impact (animal to human interface under specific epidemiological conditions).

Two goats were positive for *S.* Typhimurium and *S.* Chester. *S.* Typhimurium has a greater potential of causing human salmonellosis relative to other serotypes (Sarwari et al., 2001). This is particularly important in smallholder farms as the animals are in close contact with humans. A study in Australia tested for the presence of *Salmonella* in goats, and relatively lower prevalence of *S.* Typhimurium (13%) and S. Chester (11%) were observed as compared to *S.* Saintpaul, which had a prevalence of 31% (Duffy, Barlow, Fegan, & Vanderlinde, 2009).

Some of the serotypes detected in this study include *S.* Schwarzengrund, *S.* Pomona, *S.* Sambre, *S.* Aarhus, *S.* Cardoner and *S.* Senftenberg. S. Schwazerngrund was isolated from chicken (3.3%) and a pig (3.3%). In USA, S. Schwazerngrund has been linked to outbreaks of human infections through handling of pet food (Behravesh et al., 2010).

In this study, *S.* Senftenberg was isolated from a pig. *S.* Senftenberg is a common serovar worldwide including South Africa (Kidanemariam et al., 2010). *Salmonella* Sambre was isolated in chicken. There is limited literature about S. Aarhus, S. Sambre and *S.* Cardoner. The occurrence of these serovars in food animals owned by smallholder farmers suggests that free-range animals in South Africa may be a source of unique serotypes. The occurrence of unexpected *Salmonella* in this study could indicate that food animals raised by smallholder farmers could be an important source of novel isolates. These could be important pathogens, hence they must be further characterised.

Isolation of diverse *Salmonella* serovars among food animals highlights the need for multisectorial interaction to prevent the spread of these bacteria from animals and animal products to humans. Implementation of this 'one health' approach requires forging partnerships among stakeholders in animal and human health, environment and ecosystems personnel and policy makers (Centers for Disease Control, 2010; European Union External Action, 2011; Public Health Agency of Canada, 2009; Vandermissen and Welburn, 2014). Developing national partnerships similar to The Human and Animal Infections and Risk Surveillance (HAIRS) group in the United Kingdom would be beneficial (Vandermissen and Welburn, 2014). Such an approach will provide a robust national system for preventing proliferation of zoonotic and emerging diseases through optimized surveillance.

The variations in *Salmonella* spp proportions and serovars among different geographical areas highlights the need for surveillance programmes that will generate information necessary to implement relevant control measures in order to protect consumers from possible risk of infection from contaminated food (Office International des Epizooties (OIE) Terrestrial Animal Health Code, 2015). A comprehensive programme covering the entire food value chain continuum from 'farm to fork' is important for *Salmonella* control. The initial but imperative step in the control or reduction strategy of *Salmonella* infection due to contaminated eggs and poultry meat should be at the farm level (OIE Terrestrial Animal Health Code, 2015, chap. 6.5). This would entail testing of feed and healthy chicken that may be potential asymptomatic carriers. The national programme for *Salmonella* surveillance and control among animals could also be adopted from the OIE Terrestrial Animal Health Code (2015) and European Union documents such as 'Guidelines for the drafting of EU co-financed programmes for monitoring and eradication of zoonotic *Salmonella* in certain poultry populations' document. Initially, serovars such as *S.* Enteritidis, and *S.* Typhimurium could be targeted. The number of sampling visits for the different animal species will be determined using estimated national *Salmonella* prevalences and risk analysis data. The national programme would recommend serotyping of all *Salmonella* isolates, and assess these bacteria for antimicrobial resistance. In addition, a vaccination programme (based on prevalence rates) should be developed as part of the *Salmonella* national surveillance programme. The *Salmonella* control programme would include culling of positive animals that incorporates the cost implications prior to culling of animals. Laboratory diagnosis of *Salmonella* should be strengthened. In addition, participation in proficiency testing schemes should be compulsory for all laboratories involved in *Salmonella* diagnosis. Furthermore, Good Agricultural Practice and Hazard Analyis Critical Control Points (HAACP) approaches to food safety are crucial for prevention and control of *Salmonella* spp in poultry.

In this study, *Salmonella* enterica subspecies *salamae*, also known as *Salmonella* II, was isolated in chickens (n = 5) and a pig from Limpopo province. *Salmonella* II is commonly found in cold blooded animals (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). A study conducted in Australia found that the most frequently isolated *Salmonella* in commercially produced broiler chickens was *Salmonella* II (Duffy, Dykes, & Fegan, 2012).

3.3. Antimicrobial susceptibility testing

All *Salmonella* isolates were sensitive to cefotaxime, enrofloxacin, florphenicol and polymyxin B. Most of the *Salmonella* isolates were resistant to at least one antibiotic (66.7%; n = 20) 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%). No multi-drug resistance (resistance to ≥3 antimicrobial classes) was observed. Table 2 summarises the resistance profiles of the isolates. *S.* Techimani isolates showed co-resistance to trimethoprim/oxytetracycline (n = 2) and trimethoprim/ampicillin (n = 1).

In South Africa, the issue of antimicrobial resistance is outlined

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Table 2
Resistance profiles of *Salmonella* from this study.

Geographical origin	Animal species	*Salmonella* serovar (n)	Resistance (n)
KwaZulu Natal	Goats	*S.* Chester (1)	MY
		S. Typhimurium (1)	MY
	Chicken	*S.* Cardoner; (1)	MY
		Untypeable (1)	MY
Limpopo	Chicken	*S.* Sambre (1)	MY
	Chicken	*S.* Schwarzengrund (1)	MY
	Chicken	*Salmonella* II (5)	MY
	Pigs	*S.* Schwarzengrund (1)	MY
		Salmonella II (1)	MY
		Untypeable (3)	AMP/MY
		S. Aarhus (1)	MY/W
		S. Pomona (1)	MY/W
		S. Techimani (5)	MY/W
		S. Techimani (1)	MY/N/OT/W
		S. Techimani (1)	MY/N/W
Eastern Cape	Pigs	Untypeable (2)	AMP/MY/W
		S. Senftenberg (1)	K/MY/N
		S. Techimani (1)	MY/OT/W
		S. Techimani (1)	MY/OT

in The South African Antimicrobial Resistance Strategy Framework (Mendelson & Matsoso, 2015). The initiatives include Global Antibiotic Resistance Partnership (GARP) in South Africa (GARP-SA), South African Antibiotic Stewardship Programme (SAASP), Infection Prevention and Control and National Core Standards (IPC and NCS), and Expanded Programme on Immunization (EPI). Antimicrobial resistance is an important 'one health' challenge because it affects animal health, human health and the environment, hence it is imperative to develop a national plan that specifically targets the use of antibiotics in animal production. On-the-farm solutions to curbing *Salmonella* spp antibiotic resistance among goats, pigs and chicken among smallholder farmers could be guided by activities of the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI). Antibiotic resistance could be minimised through reducing the amount of antibiotic use by improving the general health and welfare of animals (European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI). This could be done through improving management, biosecurity and education and training of personnel including farmers, farm workers and veterinarians (EIP-AGRI). In addition, particular 'alternatives to antibiotics' such as breeding and feeding using specific approaches (control) of feed supplies and vaccination could alleviate antimicrobial resistance (EIP-AGRI). Furthermore, enhanced information dissemination and changing the behaviour and attitudes of stakeholders that are involved in animal production is important for curbing antimicrobial resistance (EIP-AGRI).

This study illustrated that pigs, chickens and goats owned by smallholder farmers are a source of diverse *Salmonella* serovars, including unexpected isolates that were not reported previously in South Africa. The diverse *Salmonella*s serovars could be a source of disease burden among animals and humans. Despite relatively low prevalence, the high proportion of antimicrobial resistant *Salmonella* spp isolated from food animals highlights the need for prudent use of antibiotics. These findings highlight the need for robust national surveillance and control programmes for *Salmonella* and antibiotic resistance in animal production as this will make a positive contribution to the 'one health' initiative.

4. Limitations of the study

This study did not establish whether there is a clear relationship between antibiotic use and antibiotic resistance in pigs, goats and chicken in the study areas. It will be important to obtain data on antibiotic use among South African smallholder farmers in future.

Declaration of conflict of interest

The authors declare no conflict of interest, either personal or financial.

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