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Full Length Research Paper

Distribution of *SPV* genes, plasmid profiles and pulsotypes of *Salmonella enteritidis* isolates of animal and human origins in selected locations of Zimbabwe

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A total of 49 *Salmonella enteritidis* isolates identified by culture and biochemical tests were confirmed using multiplex polymerase chain reaction (PCR). The isolates were grouped into two, those with *Salmonella* plasmid virulence (*spv)* gene and those without the *spv* gene. Plasmid and pulsed field gel electrophoresis (PFGE) analysis were performed on both groups. Of the 49 *S. enteritidis* isolates identified, 39 possessed a *spv* gene. Moreover, of the 39 isolates possessing the *spv* genes, 32 were from animals and 7 from humans. Those isolates without the *spv* genes comprised of 5 isolates from humans and 5 from animals. Plasmid analysis discriminated the isolates with the *spv* genes into 11 profiles and isolates without *spv* gene into 3 profiles. PFGE discriminated *S. enteritidis* isolates into 10 pulsotypes (9 from animals and 1 from the human outbreak strains). There was no correlation between the pulsotypes or plasmid profiles with the geographical location of isolate. However, there was a link between strain type and source of isolate. The study was performed to characterize *S. enteritidis* strains using molecular typing techniques and determine their geographical distribution in Zimbabwe. The information obtained can give background information for further studies on *Salmonella* epidemiology in Zimbabwe.

Key words: *spv, Salmonella enteritidis,* pulsotypes, plasmid profiles, multiplex polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE).

INTRODUCTION

Salmonella enteric subspecies *enterica* serovar *enteritidis* is one of the major causative agents of diarrhoea in humans, and is associated with the ingestion of contaminated animal products such as beef, poultry and poultry products (Simango and Mbewe, 2000). In Europe, *S. enteritidis* is involved in 80% of *Salmonella* food poisoning cases (Nygård et al., 2004). It was noted in a survey on infected adults that human immunodeficiency virus (HIV) infected and acquired immunodeficiency syndrome (AIDS) patients are most prone to non-typhoidal salmonellosis, with 35% of HIV-infected adults in Africa documented blood stream infections (Hohmann, 2001). In Zimbabwe, there is limited documented and published data on *Salmonella* epidemiology and pathogenesis in both animals and humans. There is therefore a need to study and document S*almonella* virulence genes associated with salmonellosis in both humans and animals.

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Plasmids

A naturally-occurring plasmid is a circular,

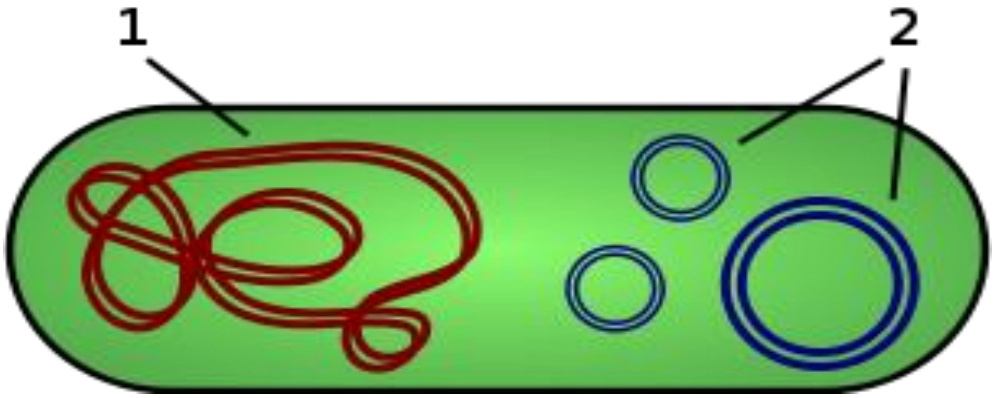

Figure 1. General bacterial cell structure showing 1-chromosomal DNA and 2-plasmids (Berg et al., 2007).

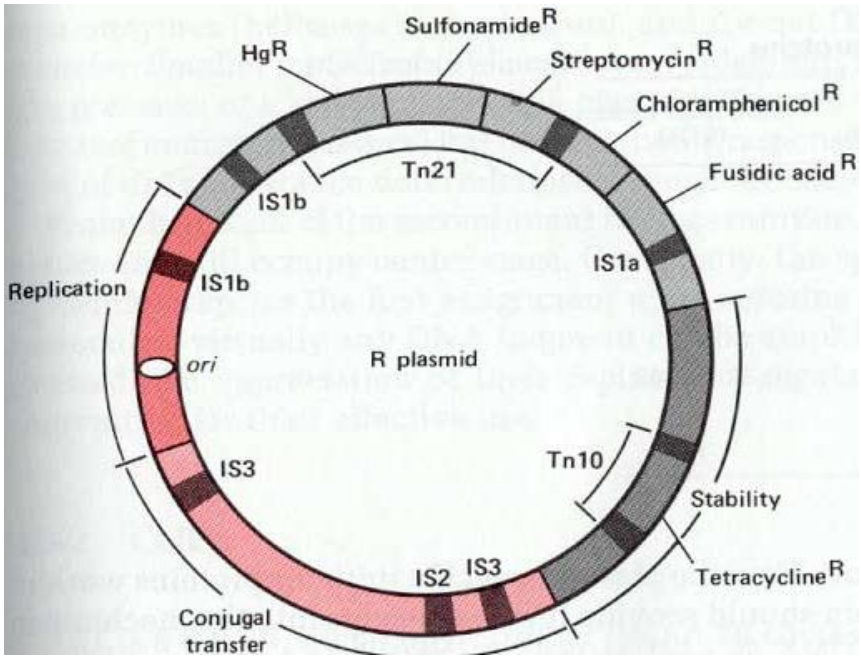

Figure 2. R-plasmid (Berg et al., 2007).

extrachromosomal, double stranded DNA (dsDNA) molecule that is found in bacteria and is capable of autonomous replication. Plasmids can range in size from 2 kilo bases (Kb) to 1000 Kb. Furthermore they can occur in a supercoiled form, although some can occur in a linear fashion. Plasmids help bacteria to adapt to their surrounding (Berg et al., 2007, Persing et al., 2004). Figure 1 shows the general structure of bacteria with plasmids. Plasmids can occur naturally; for example R-Plasmids which are large conjugative plasmids that carry genes which code for more than one antibiotic resistance. R-Plasmids also code for their own replication, their own conjugal transfer and have mobile genetic elements (Berg et al., 2007). Figure 2 shows the general structure of an R- plasmid. This is just an example amongst hundreds of plasmids existing in nature. Another naturally occurring plasmid is the Tumour Inducing (*Ti*) plasmid found in *Agrobacterium tumefaciens* which infect dicotyledonous plants causing unregulated cell division resulting in tumour formation.

Cloning plasmids however are much smaller units with three basic elements, namely, a cloning site also known as a polylinker or multiple cloning site, an origin of replication (ori) and a selectable marker. The multiple cloning site is where unique restriction enzymes cleave. The origin of replication is where replication initiates, while the selectable marker is commonly a gene for antibiotic resistance (e.g. the ampicillin resistance gene). Typical examples include pBR322 plasmids and more recently the pUC series of plasmids. Figure 3 shows the

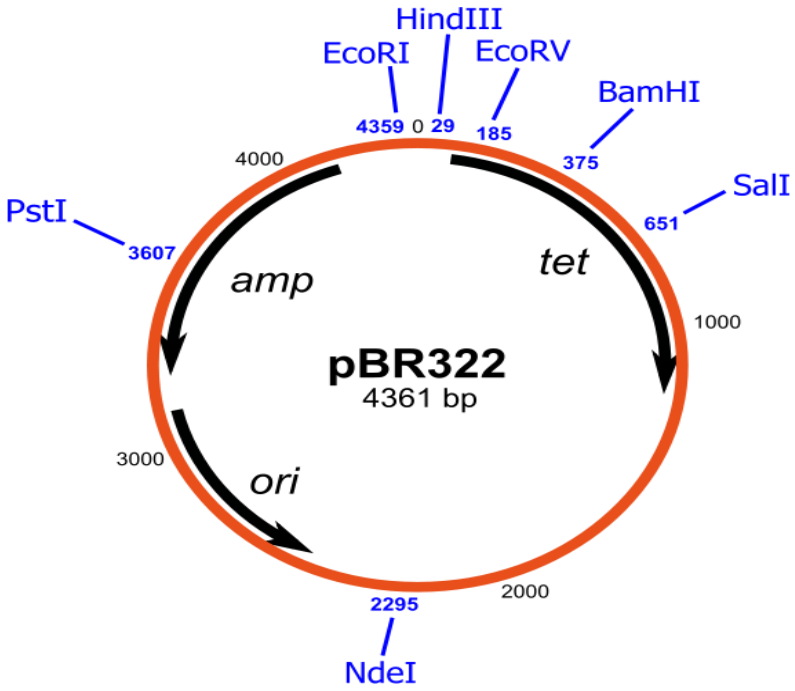

Figure 3. pBR322 plasmid (Berg et al., 2007).

structure of the pBR322 plasmid.

In nature some plasmids can be transferred from one bacterium to the next through conjugation. They have to encode all of the protein functions that are necessary for mobilisation and transfer from a host to a recipient cell. If this characteristic is lacking in a plasmid co-existing with another plasmid possessing these characteristics and present in the same cell, then the former plasmid can also be mobilized. This function is dependent on the presence of specific sequences that are recognised by the proteins encoded by the self-mobilising plasmid (Berg et al., 2007). The ability of plasmids to be transferred from one bacterium to another contributes to the spread of drug resistance in bacterial species (Rychlik et al., 2005). This is why an entire colony of bacteria may become immune to a particular drug, for example kanamycin. This characteristic has been exploited in genetic engineering in order to identify recombinant (Berg et al., 2007). Similarly the plasmid copy number can be manipulated to over-express the gene of interest (Berg et al., 2007). For example the copy number in the pUC series of plasmids is 500 per cell.

spv genes

Common features of *Salmonella* pathogenesis include the inducement of the host intestinal epithelial cells to take up the organism in a *Salmonella*-containing vacuole (SCV) and the ability to manipulate the intracellular trafficking of the vacuole to promote survival and replication of the pathogen. The whole invasion process requires the type three secretion system (TTSS or T3SS) encoded in the *Salmonella* pathogenicity island-1 (SPI-1) locus (Guiney and Fierer, 2011). Non-typhoid *Salmonella* associated with extra-intestinal infections carry an additional region/locus called *Salmonella* plasmid virulence (*spv*) region located on the virulence plasmid (Ridley et al., 1998). *Salmonella* virulence plasmids are heterogeneous in size and can range from 50 to 90 kb but all share a 7.8 kb (*spv*) region (Boyd and Hartl, 1998; Rotger and Casadesus, 1999).

Virulence plasmids have been identified in some invasive serovars such as *S. typhimurium, S. enteritidis, S. dublin, S. cholerasuis, S. gallinarum, S. pullorum* and *S. abortusovis*. Some of these virulence plasmids are species-specific, for example the 50 kb pSCV plasmid of *S. cholerasuis*, 94 kb pSLT plasmid of *S. typhimurium* and 55 kb plasmid of *S. enteritidis* (Rychlik et al., 2005).

The role of *spv* genes in *Salmonella* pathogenesis

The *spv* gene plays an important part in the clinical syndrome due to *Salmonella* infection. The *spv* region is a 7.8kb conserved region of *Salmonella* virulence plasmids required for multiplication of the bacterial pathogen in the reticuloendothelial system of the host. Other loci of the plasmid such as the fimbrial operon, *pef,* the conjugal transfer gene, *tra*T and the enigmatic *rck* and *rsk* loci may play a role in other stages of the infection process (Boyd and Hartl, 1998; Rotger and

Casadesus, 1999).

Basically, *Salmonella* causes three types of disease in humans which are gastroenteritis, usually due to *S. Typhimurium*, enteric fever as a result of *S. Typhi* and non-typhoid extra-intestinal disease with bacteremia caused usually by S. *Enteritidis*. It has been proven that each clinical syndrome requires distinct sets of virulence genes (Guiney and Fierer, 2011). *Salmonella* isolates do differ in the assemblage of their virulence traits. Research has indicated that *spv* locus is "strongly associated with strains that cause non-typhoid bacteremia, but are not present in typhoid strains" (Guiney and Fierer, 2011).

Typing techniques

As stated by Boyd and Hartl (1998), the traditional typing techniques involve the use of selective culture media, biochemical and serological tests (Pan and Liu, 2002). These traditional methods do not give differences of organisms beyond species or serotype levels. The advent of molecular techniques has made it possible to identify differences within the same serogroup. Consequently traditional typing methods should always be complemented by molecular typing techniques to enhance their utility (Namoos et al., 1994).

In this study, multiplex polymerase chain reaction (multiplex PCR) (Pan and Liu, 2002), pulsed- field gel electrophoresis (PFGE) and plasmid profiling (Prager et al., 2003) were used to type *S. Enteritidis* serovars at molecular level.

Multiplex PCR

Multiplex PCR is a variant of the ordinary PCR which deals with the use of more than one primer per reaction to simultaneously amplify many target regions. It is usually applied in the analysis of multiple markers, detection of pathogens or genetically modified organisms (GMOs) or for microsatellite analyses. The setting up of multiplex PCR requires intensive optimisation of the procedures. Multiplex PCR has the advantage that each amplicon provides an internal control for the other amplified fragment. This is because failure of one fragment to amplify while the others amplify will be an indicator that the reaction has not failed (Foley et al., 2007). It can be used as a diagnostic tool or confirmatory tool after serotyping (Pan and Liu, 2002).

Plasmid profiling

This study used plasmid profiling as one of the typing tools. Plasmid profiling of an organism involves the isolation of plasmid DNA from an organism of interest, followed by the separation of these molecules based on their size by agarose gel electrophoresis. Although plasmids can be transferred between organisms, their presence or absence can be an important epidemiological marker (Ridley et al., 1998). In general, plasmids of 10 kb or less occur in 10% of *Salmonella* field strains (Ridley et al., 1998). The function of these low molecular weight plasmids were previously not known to encode distinguishing traits and were mostly used in molecular typing as opposed to the high molecular weight ones that were known to encode distinguishing traits. It has, however, been discovered that these low molecular weight plasmids code for retron reverse transcriptase and tend to influence phage resistance (Rychlik et al., 2001).

PFGE

PFGE was once considered the golden method of molecular typing, but is now being superseded by more versatile variable number tandem repeats (VNTRs) and multi locus sequence typing (MLST). From their research on the stability of multi-locus variable number of tandem repeats in *S. Typhimurium*, Hopkins et al. (2007) discovered that VNTR was more stable and provided better discrimination compared to PFGE (Namoos et al., 1994; Hopkins et al., 2007). PFGE is essentially the comparison of large genomic DNA fragments after digestion with a restriction enzyme. It has been shown to be highly effective in epidemiological studies involving a variety of bacteria including *Salmonella* serovars *enteritidis* and *typhimurium* (Karim and Islam, 2002). Since the bacterial chromosome is typically a circular molecule, digestion with a rare-cutting restriction enzyme such as *Not I* yields several linear molecules of DNA. The rationale is that when comparing two strains that are clonal, the sites at which the restriction enzymes act on the DNA and the length between these sites would be identical. Therefore, after digestion of the DNA and electrophoresis through an agarose gel, if the DNA banding patterns between any two isolates is identical, then these isolates are considered the same strain, and referred to as being monomorphic. On the other hand, if two isolates are not the same strain, then the sites at which the restriction enzymes act on the DNA and the length between these sites would be different; thus their DNA banding patterns will be different (Basim and Basim, 2001) and are therefore referred to as being polymorphic.

The preparation of genomic DNA suitable for PFGE begins by lysing bacteria that are embedded in agarose blocks (Karim and Islam, 2002). After multiple washes, the DNA within the agarose is digested with restriction enzymes and subjected to electrophoresis using PFGE. PFGE differs from conventional agarose gel electrophoresis in that the orientation of the electric field across the gel is periodically changed in contrast to being unidirectional and constant in standard electrophoresis. The variability in the electric field allows PFGE to resolve the very large fragments (>600 kb) associated with this

analysis. The banding patterns obtained are the pulsotypes used in typing to identify the different strains of organisms (Basim and Basim, 2001).

The main purpose of this study is to characterize *S. Enteritidis* strains using molecular typing techniques and determine their geographical distribution in Zimbabwe. This was aimed at providing basic information for the development of a molecular typing system for *Salmonella* that will improve the control of *Salmonella* infections in wildlife, domesticated animals and humans. The study is therefore targeted for scientists with interest in zoonotic research with special reference to salmonellosis in animals and humans. The other target groups are farmers and human health workers interested in the prevention, diagnosis, epidemiology and control of salmonellosis.

MATERIALS AND METHODS

Sample collection, *Salmonella* isolation and serotyping

The sampling sites were Mutare, Bulawayo, Kariba and Harare. Animal *Salmonella* strains were isolated from bovine, avian (chicken and ostriches) and crocodiles. Swabs and organs from animals were taken from various farms to the Central Veterinary Laboratory (CVL). The swabs and organs were put in selenite broth, an enrichment media for *Salmonella*, soon after receiving. The samples in selenite broth were immediately stored at 4°C till they were cultured (but not stored for more than a week in cold room). *Salmonella* strains of human origin that were collected as isolates were outbreak strains collected from Harare hospital, a referral hospital and from routine Bacteriology work from Central Veterinary Laboratory (CVL), bacteriology section. The CVL human Isolates were isolated from anal swabs in selenite broth. The anal swabs were taken from employees of Colcom pig and Kariba crocodile abattoirs, which are meat processing companies in Zimbabwe.

The samples in selenite broth were incubated at 37°C overnight before culturing on blood agar (BA) and MacConkey agar, and then incubated overnight at 37°C. Suspected *Salmonella* colonies were further cultured on xylose lysine deoxycholate (XLD) agar. Triple sugar iron (TSI), lysine-decarboxylase and urease were the biochemical tests used to confirm XLD *Salmonella* suspected colonies. The *Salmonella* strains were serotyped in accordance with the Kauffmann-White scheme based on one serotype-one species concept on the basis of somatic (O) and flagella (H) antigens (Brenner et al., 2000) to identify the serovars.

Multiplex PCR assay

The DNA used for this assay was extracted using hexadecytrimethyl ammonium bromide (CTAB) DNA extraction method (Rafael at genetics.med.utah.edu). Multiplex PCR was performed on isolates identified by serotyping technique as *Salmonella* both of human and animal origin. Multiplex PCR was carried out using the following primer pairs: ST11 (gccaaccattgctaaattggcgca) / ST 14 (ggtagaaattcccagcgggtactgg) which amplifies a randomly cloned sequence of 429 bp, specific for the genus *Salmonella*, SEFA 2(gcagcggttactattgcagc) / SEFA 4 (tgtgacagggacatttagcg) which amplifies the *Salmonella enteritidis* fimbril antigen gene, 310 bp long and S1(gccgtacacgagcttataga) / S4 (acctacaggggcacaataac) which amplifies the *Salmonella* plasmid virulence gene (*spv),*250bp long (Pan and Liu, 2002; Akbarmehr, 2011).

The following reagents' final concentrations were used in the PCR reaction: 1.5 mM magnesium chloride; 1X PCR buffer; 0.2 mM deoxynucleotide triphosphates (dNTPs); 0.2 µM primer; 0.125 U *Taq* polymerase enzyme; 2 µl sample DNA. The mix was made up to required volume using sterile double distilled water. The PCR was run using the following program: 1 cycle of pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s; annealing at 56°C for 90 s and extension at 72°C for 30 s. Finally, 1 cycle of final extension stage at 72°C for 7 min was performed before storage at 4ºC. The amplicons were loaded on 1% agarose gel stained with ethidium bromide solution of a final concentration of 0.5 µg/ml and subjected to electrophoresis in 0.5X Tris-borate EDTA (TBE) electrophoresis buffer at 90V. A 100 bp molecular weight marker was used to verify the band sizes produced. After electrophoresis, the gel was viewed under a UV transilluminator and photograph taken using the Kodak Gel Logic 100 imaging system supplied by Eastman Kodak Company, Rochester, New York, USA.

Plasmid profiling

All isolates identified as *S. Enteritidis* were genotyped by the plasmid profiling technique. Plasmid extraction was performed using modified Kedo and Liu alkaline lysis method (Kedo and Liu, 1981). The extracts were run on 0.7% agarose gel stained with ethidium bromide solution of a final concentration of 0.5 µg/ml, using V517 and 39R861 *E. coli* plasmids as markers. After electrophoresis, the gel was viewed under a UV transilluminator and photograph taken using Kodak gel logic 100 imaging system. The gel photographs were analysed to identify the different profiles obtained and relate the profiles to host and geographical location.

Pulsed field gel electrophoresis (PFGE)

PFGE was done according to modified E. J Threlfall protocol (Ridley et al., 1998) on the 49 strains which were grouped according to plasmid fingerprints. Plugs were prepared by mixing 20 µL of proteinase K (Invitrogen 400 U/ml), 500 µL of sample suspension of 0.5 to 0.55 with O.D at 600 nm wavelength and 500 µL of 2% low melting point (LMP) agarose. The mixture was set in plug moulds for 10 min at 4°C. The plugs were removed from moulds transferred to sterile glass tubes with 2 ml cell lysis buffer and 3 U (7.5 µL) of proteinase K before incubating for 2 h at 55°C in a shaking water bath. The plugs were transferred to 50 ml sterile tubes and washed twice for 10 min in a shaking water bath with sterile distilled water that was preheated to 55°C. The plugs were cooled to room temperature in TE buffer then left in the TE buffer at 4°C for at least a week before restriction digestion was performed. The restriction digestion was performed by incubating each plug at 37°C overnight using 20 units of *XbaI* restriction enzyme.

Two thirds of each of the digested plugs was loaded on a 1% ordinary agarose gel. On the gel, the sample plugs were sandwiched by a commercial PFGE marker. The PFGE programme was run for 22 h at 6 V/cm (200 V) and 14°C with switch time of 2 to 64 s and a 120° linear corner using the Biorad PFGE apparatus. A volume of 2.5 L of 0.5X TBE buffer was used during electrophoresis as the electrolyte. The gel was stained in 0.5 µg/ml ethidium bromide solution for 20 min and then de-stained in 1 L of distilled water for 30 min. After electrophoresis, the gel was viewed under a UV transilluminator and photograph taken using the Kodak Gel Logic 100 imaging system supplied by Eastman Kodak Company, Rochester, New York, USA. The different pulsotypes were identified and used for analyses to determine isolate differences.

RESULTS

Sample collection, *Salmonella* isolation and serotyping

A total of 214 samples were collected from animals for isolation of *Salmonella*. Of the 214 samples collected, 35 (16.33%) were positive for *Salmonella* after serotyping and were all from ostriches. In addition to these isolates, 58 *Salmonella* strains were collected as isolates from Mutare Veterinary Laboratory (MVL) and 57 from Central Veterinary Laboratory (CVL). The animal *Salmonella* isolates collected from CVL were from Victoria Falls (crocodiles n = 4), Kariba (crocodile n = 12), Binga (crocodile n = 10), Harare (avian n = 23, caprine n = 1), Chinhoyi (bovine n = 6) and Norton (porcine n = 1). All in all 150 isolates were of animal origin.

For human isolates, a total of 135 *Salmonella* isolates were collected of which 117 /135 were from Harare Hospital and 18/135 were from CVL. The total *Salmonella* isolates used in this study from humans and animals was therefore 283. Table 1 and Figure 4 show the distribution of isolates used in this study.

Multiplex PCR

Two of the 35 isolates from ostriches that had been shown to be *Salmonella* positive by serotyping were dropped after Multiplex PCR failed to confirm their status as *Salmonella*, bringing them to 33 (15.42%) of the 214 samples analysed. All the 283 *Salmonella* isolates from animals and humans were subjected to Multiplex PCR assay. Multiplex PCR confirmed 14 bovine *Salmonella* isolates collected from Mutare Veterinary Laboratory to be *S. Enteritidis.*

In addition to 15 /57 *Salmonella* isolates (26.32%) collected from CVL, all isolates from chicken sources were confirmed *S. Enteritidis* strains. For human isolates 12 (8.89% expressed as a percentage of the 135 human isolates) from CVL and 8 (5.93% expressed as a percentage of the 135 human isolates) from Harare hospital, were confirmed to be *S. Enteritidis*. Overall, 49/ 282 (17.38%), were confirmed *S. Enteritidis*. This is illustrated in Figure 5.

Multiplex PCR was also in a position to group *S. Enteritidis* strains into 2 groups: those with *spv* genes and those without *spv* genes. Of the 49 *S. Enteritidis* isolates confirmed, 39 (79.59%) had the *spv* gene as shown by the presence of a band of approximately 250 bp of which 31 (79.49%) were of animal origin and 8 (20.51%) were of human origin (all from outbreak), and 10 (20.41%) did not have *spv* gene of which 5 (50%) were of animal origin (all from Harare) and 5 (50%) were of human origin (4 from Kariba and the other one with an unknown source.

Figures 6 and 7 shows the percentage distribution of *S. Enteritidis* strains with and without *spv* genes and the multiplex PCR for the human group isolates.

Plasmid extraction and profiling

All the 49 confirmed *S. Enteritidis* strains were subjected to plasmid extraction and profiling. All the 8 outbreak strains of human origin, with and without the *spv* gene, shared a common heavy plasmid of approximate size of 54 Kb. Of the 32 strains from animals, 12 (37.5%) lacked the heavy plasmid despite them carrying the *spv* gene (Figure 8). A total of 11 plasmid profiles have been identified from strains with *spv* gene and 3 from the strains without *spv* gene. Five out of seven (71.4%) *S. Enteritidis* isolates from ostriches shared the same profile. Of the 5 isolates, 3 (42.8%) were from Bulawayo province and 2 (28.6%) from Harare province. Sharing the same profile with ostriches were 2 avian isolates from Harare province. Figure 9 shows the generated plasmid profiles.

Pulsed field gel electrophoresis

PFGE identified 10 pulsotypes of which pulsotype 1 was from clinical human isolates from 2005 salmonellosis outbreak and the other 9 were for animal isolates (Figure 10). Of the bovine isolates from Mutare, 8/13 (61.5%) were identified under one profile using PFGE and 5/13 (38%) of the same isolates from Mutare had the same profile (Figure 11). Generally, strains obtained from animals produced more profiles compared to those isolated from humans.

DISCUSSION

This study was aimed at the identification of *spv* genes in *S. Enteritidis* and the relationship of these genes, plasmid profiles and PFGE profiles to geographical distribution and host organism of the *S. Enteritidis* isolates studied. Plasmid profiling was not in a position to split the isolates into those with *spv* genes and those without the genes. For example, some of the isolates that were observed to contain the *spv* gene after multiplex PCR analysis, lacked the heavy plasmid (54kb) associated with virulence after plasmid fingerprinting; this in an unusual finding. This finding might probably be a result of poor extractions since the traditional plasmid extraction method was used instead of the plasmid extraction kit due to limited funding. The approximate 54 kb heavy plasmid observed is in line with the 55 kb species specific virulence plasmid of *S. Enteritidis* (Rychlik et al., 2005). However, with PFGE analysis, it was possible to split the isolates into two, those with *spv* genes and those without *spv* genes. This indicates the high discriminatory power of PFGE compared to plasmid analysis technique. The fact that some of the isolates from outbreak lacked the spv gene that is usually associated with virulence might indicate that the isolates had virulence genes on the *Salmonella* pathogenicity island (SPI) locus on the chromosomal

Table 1. The distribution of *Salmonella* isolates used in the study.

Population	Location	Source	Number of isolates
From animals			148 *Salmonella* isolates from animals
	Victoria Falls	Crocodile	4
	Kariba	Crocodile	12
	Binga	Crocodile	10
	Harare	Chickens	23
		Caprine	1
	Chinhoyi	Bovine	6
	Norton	Porcine	1
	Mutare	Bovine	58
	Bulawayo North	Ostriches	33
From humans	From CVL and Harare Hospital		135 Total *Salmonella* isolates from humans
	From Harare Hospital		117 isolates from Harare Hospital
			40 group C
	-Harare		27
	-Norton		1
	-Beatrice		2
	-Gokwe		2
	-Zaka		1
	-unknown		7
			33 Group B
	-Harare		18
	-unknown		15
			8 Group D
	-Harare		4
	-Kariba		4
			35 *Salmonella* species
	-Harare		29
	-Beatrice		1
	-Chinhoyi		1
	-Kariba		4
			1 Group G
	Unknown		1
	From CVL		18 *Salmonella* Species
	-Harare		7
	-Chinhoyi		1
	-Kariba		10

DNA and not on plasmids (Guiney and Fierer, 2011).

Meanwhile, no link has been observed between the presents or absents of *spv* gene with geographical distribution of isolates. This is most probably due to migration of animals and humans from one region to another which results in the spreading of the strains

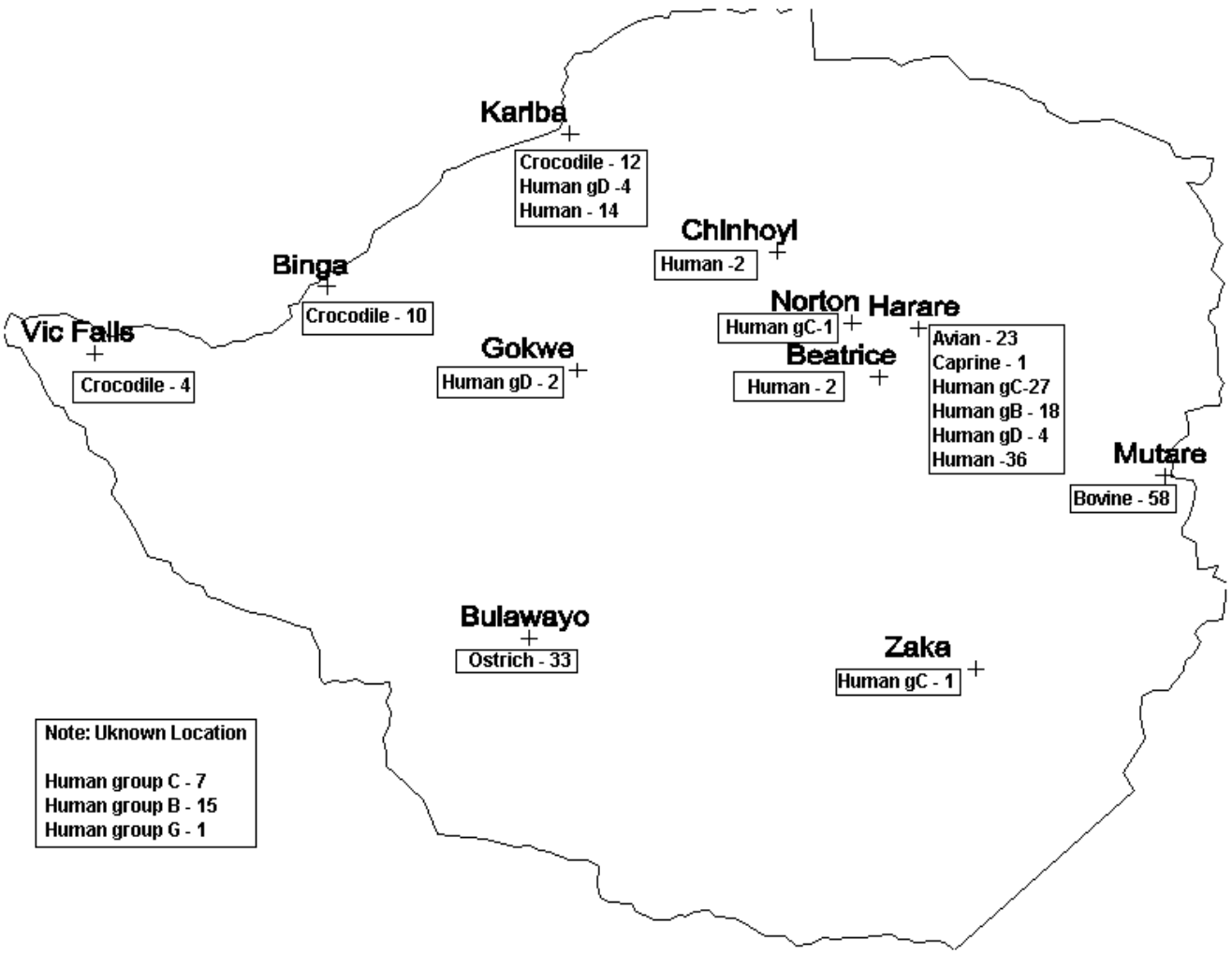

Figure 4. Map showing distribution of *Salmonella* isolates used in this study.

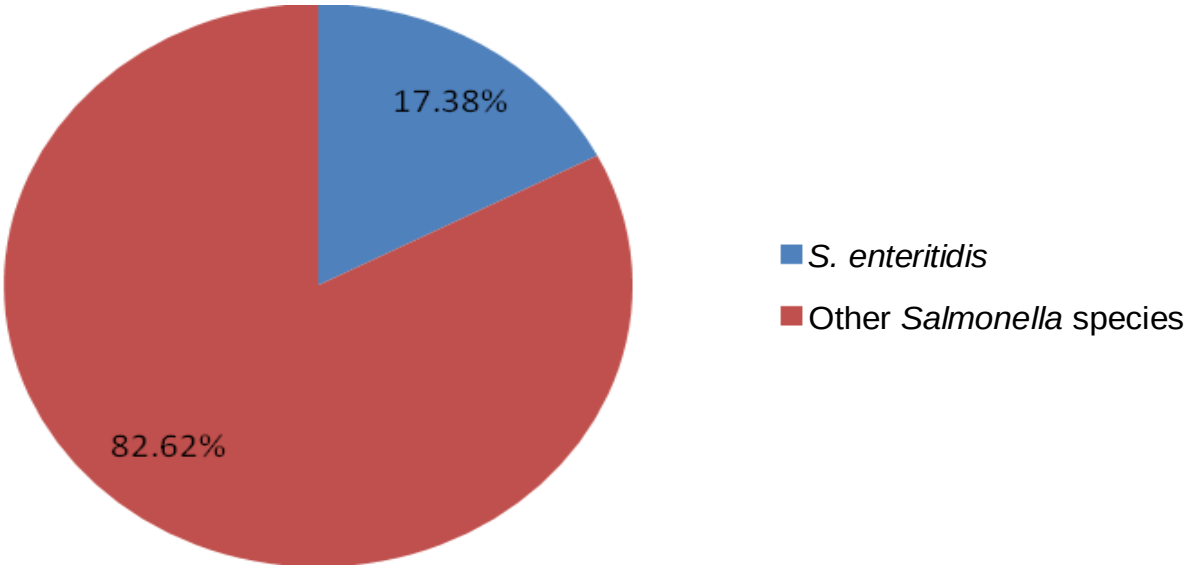

Figure 5. Percentage of *S. Enteritidis* strains confirmed from the 282 *Salmonella* isolates subjected to multiplex PCR.

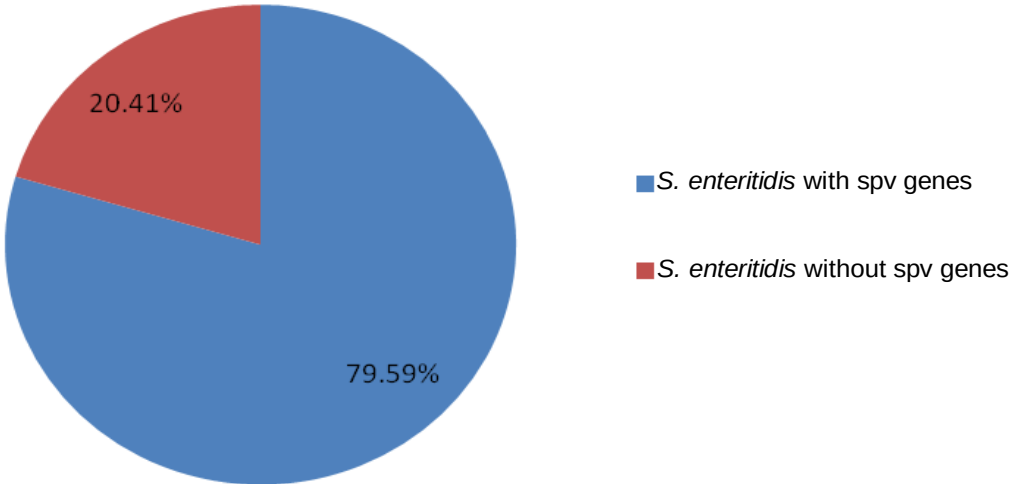

Figure 6. Percentage of *S. Enteritidis* strains with *spv* genes compared to those without *spv* genes.

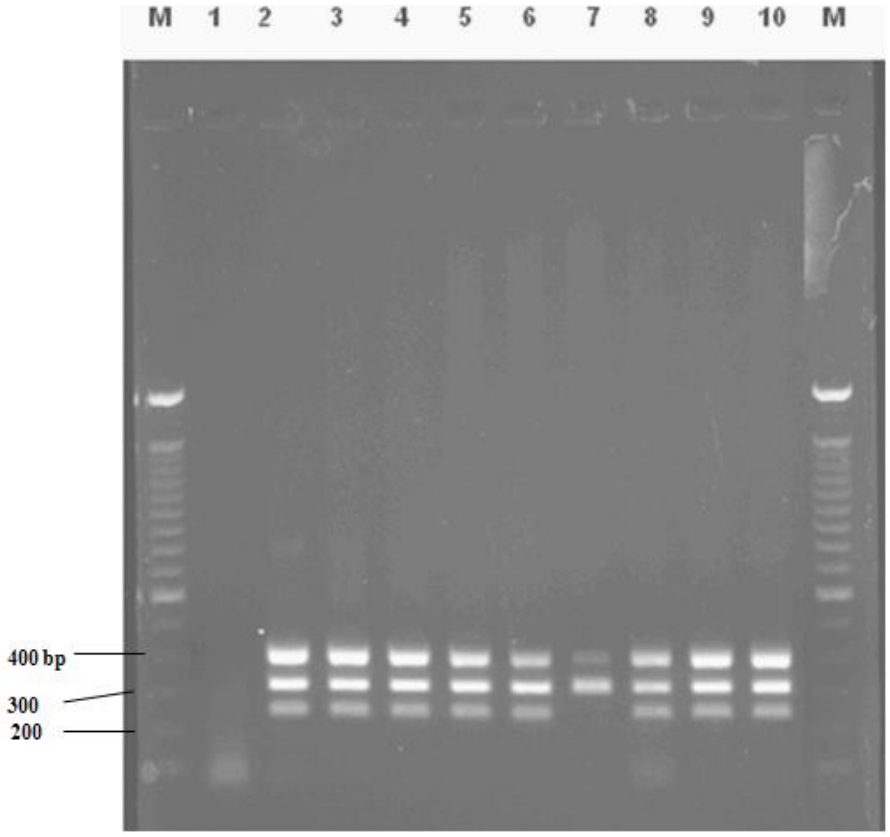

Figure 7. Multiplex PCR for human group D isolates. M is the 100 bp ladder. Lanes 1 – 10 are human group D outbreak *Salmonella* isolates from Harare Hospital.

peculiar to a region to other regions. This poses difficulties to ascertain strains to a geographical location. The results indicate relationship between *S. Enteritidis* isolate and its host. This might be an indicator of the strains that are host specific. As an example, ostriches from Bulawayo and chickens from Harare (all avian) shared the same plasmid profile. The same strains shared the same plasmid profile with clinical outbreak human isolates from Harare and Kariba. Moreover, most field strains of *S. Enteritidis* collected from farms were from healthy animals. This might be an indicator that animals are reservoirs of *Salmonella*. There is therefore a

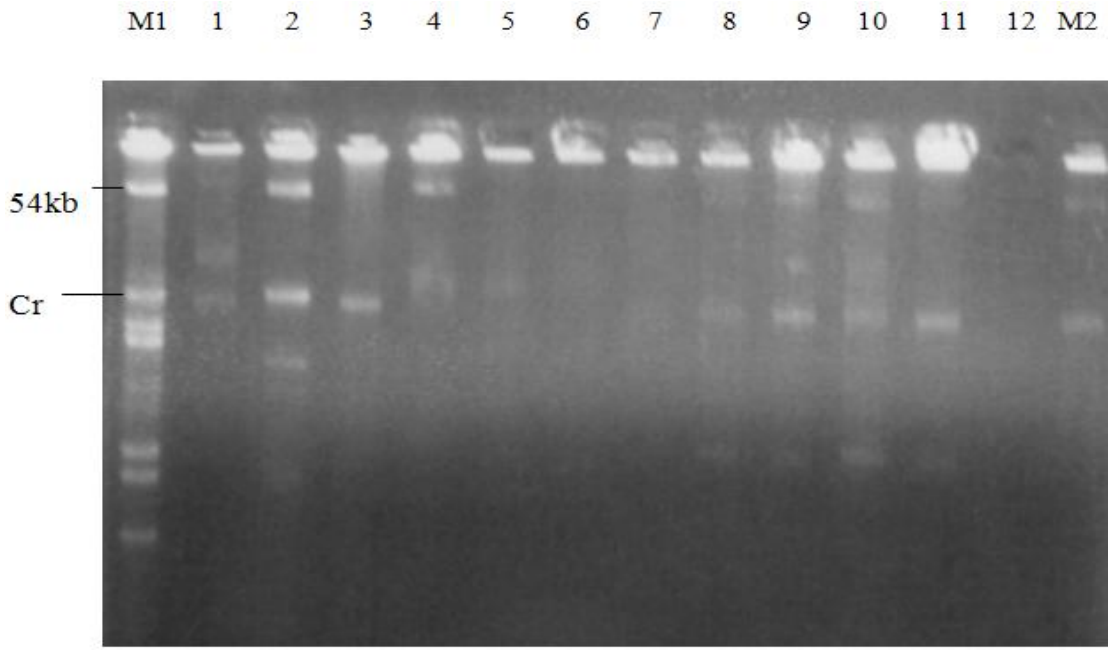

Figure 8. Plasmid profiling results of some of the *S. Enteritidis* strains of animal origin found to be possessing *spv* gene. M1 is V517 marker, M2 is 39R861 marker and lanes 1 to 12 are *S. Enteritidis* strains possessing *spv* gene.

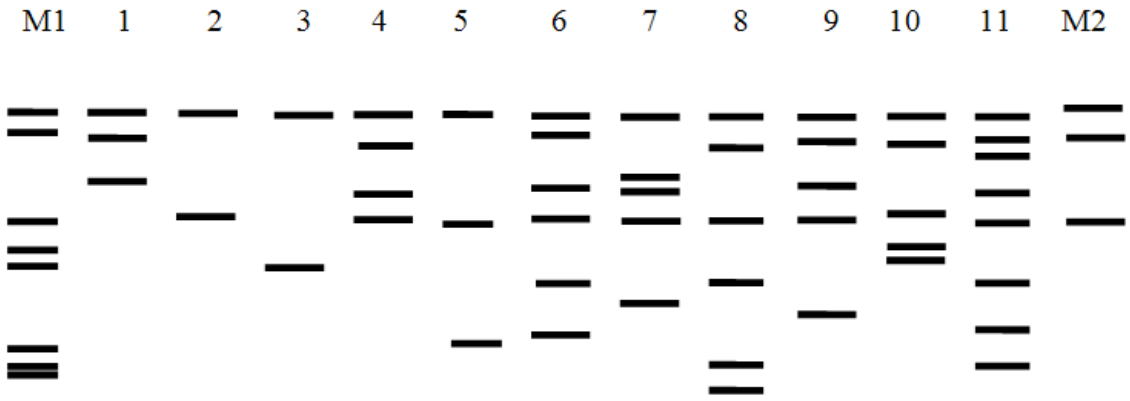

Figure 9. Combined generated plasmid profiles generated from the 49 *S. Enteritidis* strains with *spv* gene and without the gene. Lane M1 is V517 plasmid marker, M2 is 39R861 plasmid marker, 1 to 11 are *S. Enteritidis* strains.

possibility of avian strains (and other strains) being transmitted to humans through the food chain.

In plasmid profiling, 11 profiles were obtained from the 49 strains analysed. Makaya (unpublished data) obtained 5 profiles from 179 *S. Enteritidis* isolates isolated from poultry. The difference noted can be as a result of the fact that the strains used by Makaya were all from Zimbabwean poultry, while those used in this research were from humans as well as animals, stretching from poultry, ostriches, bovine and crocodile, thus resulting in a broader range of fingerprints.

The outbreak strains produced the same fingerprint after PFGE analysis, indicated as P10 in Figure 11, which might indicate that the isolates were clonal. The fingerprint can be considered as the outbreak fingerprint which can be used as a reference fingerprint if the outbreaks recur. Furthermore, it appears that the strain type of *S. Enteritidis* is not affected by the location within the host. This is indicated by the fact that the *S. Enteritidis* from humans produced the same profile regardless whether it was isolated from stool or blood. There is a high possibility that the isolates were clonal since *Salmonella*, under severe cases, can migrate to the lymph tracts which carry water and protein to the blood

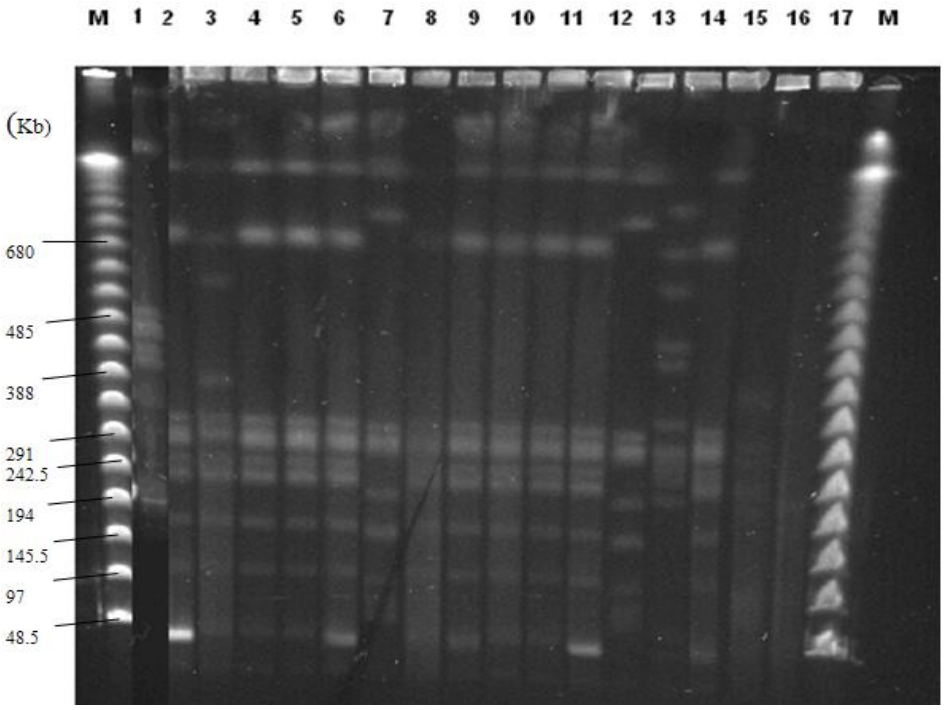

Figure 10. Animal *S. Enteritidis* pulse field gel electrophoresis of chromosomal DNA digested with *Xba*I. Lane M is the pulse field marker, 1 -17 are the *S. Enteritidis* strains.

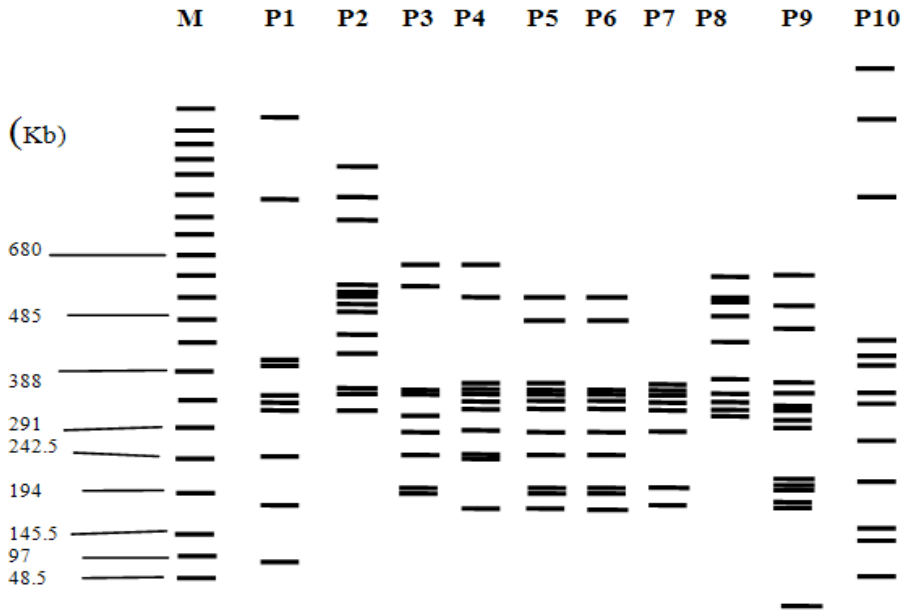

Figure 11. Pulsotypes Identified from the PFGE performed on the 35 *S. Enteritidis* isolated from animals and 8 human outbreak strains. Lane M is the PFGE marker. P1 to P9 are the identified pulsotypes from animals and P10 is the outbreak fingerprint.

(http://www.netdoctor.co.uk/health_advice/facts/salmonella.htm) and the blood itself and sometimes infect other organs (http://science.jrank.org/pages/5944/Salmonella-Causes-symptoms.html).

At the moment, there is limited documented and published literature on *Salmonella* outbreak data in

Zimbabwe. There is therefore need to put up an instrument to monitor *Salmonella* surveillances and outbreaks in Zimbabwe since *Salmonella* have been recorded as one of the deadly pathogens worldwide (Nygård et al., 2004). Statistical records and important findings should be documented and published for easy access by researchers, epidemiologists, farmers, veterinary and human health workers.

Study limitations

There is need to carry out the study with a larger sample size to authenticate the findings from the current study. The selection of animals used is not representative enough of animals in Zimbabwe since common animals like dogs, cats, horses, donkeys were not catered for. It would have been more informative if Southern blot analysis and sequencing had been used to complement the PCR methods.

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