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Detection of *inv* A virulence gene by polymerase chain reaction (PCR) in *Salmonella* spp. isolated from captive wildlife

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ABSTRACT

Salmonella possesses genes that are responsible for virulence and establishment of infection in host cells. One of such genes is *inv* A gene which is responsible for the invasion of the bacteria into host cells and is essential for full virulence in *Salmonella*. The gene is specific for members of the genus *Salmonella* and have been said to always be present in *Salmonella* spp. In this investigation, *Salmonella* spp. isolated from captive wildlife were screened for *inv* A virulence gene. Eight isolates were tested by polymerase chain reaction (PCR) assay using primers that amplify the 284 base pair fragment of the *Salmonella inv* A gene. Five out of the eight isolates were positive for *inv* A gene. They were isolates from striped hyena, lion, spotted hyena, African hawk eagle and peafowl while those from bateleur eagle, chimpanzee and another from African hawk eagle were negative. *Salmonella* isolates carrying the *inv* A virulence genetic sequence are able to invade host cells and cause infection while those without it may be unable to do that. The finding of this study also suggests that *inv* A gene may not always be present in all *Salmonella* spp.

Keywords: *Salmonella*, *inv* A, polymerase chain reaction, virulence gene, captive wildlife

INTRODUCTION

Salmonella infections occur worldwide, affecting human, different domestic and wild animals in both developed and developing countries and are a major contributor to morbidity and economic costs [1-9]. The organism is an important pathogen that shows different disease syndromes and host specificities according to their antigenic profiles [10, 11]. It can colonize and cause disease in a variety of food producing and non food producing animals [12]. The virulence of *Salmonella* is linked to a combination of chromosomal and plasmid factors, the chromosomally located invasion gene; *inv* A is thought to trigger the invasion of *Salmonella* into epithelial cells [13] while the operon (*spv* RABCD), which contains five genes and is present in plasmids commonly associated with many serotypes, determines the ability to increase the severity of enteritis and allow infection and persistence at extra intestinal sites [14].

The *inv* A gene of *Salmonella* contain sequences unique to this genus and has been proved to be a suitable polymerase chain reaction (PCR) target with potential diagnostic application [15]. This gene is recognized as an international standard for detection of *Salmonella* genus [16]. The gene is an invasion gene conserved among *Salmonella* serotypes [17].

Serotypes of *Salmonella* that do not possess the *inv* A gene are not capable of expressing *inv* ABC genes, making them unable to invade mammalian cells [18]. The gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissues [19]. This work aimed to detect the virulence gene; *inv* A by PCR in *Salmonella* isolates from captive wildlife which were confirmed using conventional tests and microbact GNB12E [20].

MATERIALS AND METHODS

Polymerase chain reaction (PCR) for *Salmonella inv* A gene detection

Isolates were subcultured on XLD agar and incubated for 24 hours at 37°C. Pure cultures were picked and inoculated into tryptone soya broth and incubated for

24 hours at 37ºC for preparation of DNA extraction. The PCR primers targeted the *Salmonella inv* A gene, which is specific to members of the genus. The primers used were:

5' GTG AAA TTA TCG CCA CGT TCG GGC AA-3' Forward
5'-TCA TCG CAC CGT CAA AGG AAC C-3' Reverse

An amplicon of 284bp was predicted, as published by Rahn *et al.* [21] and the primers were synthesized by Fermentas ™, Germany.

DNA extraction

ZR Fungal/Bacterial DNA MiniPrep™ ZRD6005 was used for the DNA extraction. The manufacturer's instructions were followed for the extraction of the DNA template from the *Salmonella* isolates. (Inqaba Biotechnical industries (Pty) Ltd., Hatfield 0028 South Africa).

***Salmonella* polymerase chain reaction**

Twenty five microlitre master mix (manufactured by Fermentas ™, Germany) composed of $MgCl_2$, Taq (*Thermus aquaticus*) polymerase, and dNTPs were mixed with 10 µl template DNA, 2.5 µl forward primer, 2.5 µl reverse primer and 10 µl nuclease free water in a PCR microtube with the following cycling parameters: initial denaturation at 95ºC for 2 minutes and 30 cycles of amplification consisting of denaturation at 94º for 30 seconds, annealing at 53ºC for 30 seconds, extension at 72ºC for 60 seconds and final extension was at 72ºC for 7 minutes. The PCR was performed in a DNA thermal cycler (Applied Biosystems, Gene Amp PCR system 9700).

At the end of the run, 10 µl of the final reaction product was separated by electrophoresis on 2% agarose gel along with 50bp DNA ladder. The products were visualized by staining with ethidium bromide, using Bioimaging system, ChemiGenius: image capture and analysis system (Manufactured by Syngene Europe, Beacon house Nuffield road, Cambridge CBITF).

RESULTS AND DISCUSSION

Out of the eight confirmed *Salmonella* isolates subjected to PCR, five were positive for *inv* A gene showing the *inv* A band as shown in the photograph of the 2% agarose gel.

The photograph showing the PCR amplification of *inv* A gene is given in Figure 1, lanes 3, 7, 8, 9 and 10 representing isolates from striped hyena, lion, spotted hyena, African hawk eagle and peafowl respectively which produced *inv* A gene bands of 284bp, this is consistent with the known band of the used positive control in lane 1.

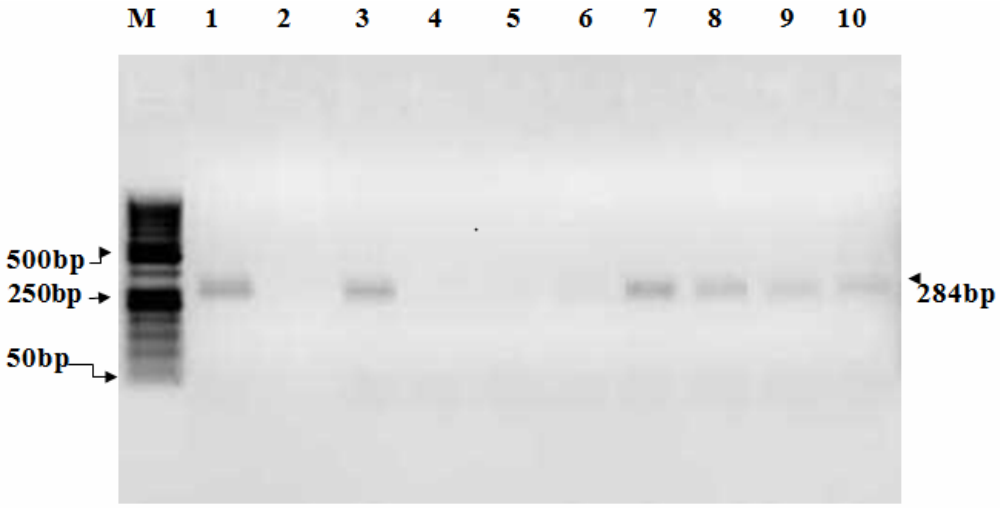

Figure 1: Photograph of agarose gel showing amplicons of *inv A* gene in eight *Salmonella* isolates tested, where M; Marker, 1; *Salmonella* positive control, 2; negative control, 3; stripped hyena, 4; bateleur eagle, 5; African hawk eagle, 6; chimpanzee, 7; lion, 8; spotted hyena, 9; African hawk eagle, 10; peafowl.

inv A gene is a chromosomic sequence which is responsible for *Salmonella* virulence especially the invasion of the host cells by the bacteria. The detection of the gene in the *Salmonella* isolated implies the organisms are virulent and will be able to penetrate host epithelia cells, causing infection. Three out of the eight confirmed *Salmonella* isolates were not positive for *inv* A gene. The absence of the gene in the confirmed *Salmonella* isolates can lead to lack of invasiveness by those isolates. This is in agreement with the report of Bacci *et al*. [22] in which *inv* A gene was detected in 62 of the 63 strains of *Salmonella* screened, they submitted that all the strains carrying that genetic sequence can potentially invade epithelial cells [22]. This implies that the isolate that do not carry the gene may not be able to invade epithelial cells and may not be virulent.

This finding is at variance with the reports of Oliveira et al. [17]; Zahreai et al. [23]; Trafny et al. [24]; Jamshidi et al [25]; Nashwa et al. [26] and, Amini et al. [27] who all detected and reported *inv* A gene in all *Salmonella* isolates they tested.

The implication of the presence of *inv* A gene in the isolates is that the organisms are actually able to cause infection in wildlife from which they were isolated, especially if host immunity is suppressed. They may also cause infection in human beings if the bacteria are contracted either from the wildlife directly or contaminated objects around them. This is especially important in animal contact settings where contacts are made with wildlife and objects around them.

CONCLUSION

Salmonella may possess *inv* A gene which is responsible for invasion of host cells and virulence. The gene may not also always be present in all *Salmonella* spp. While it may be possible to state that the species that showed the *inv* A band may be virulent, penetrate and cause infection in host cells. It could also be stated that *Salmonella* species that do not possess *inv* A gene may not be virulent and not able to invade and cause infection in host cells. Because *inv* A gene was not present in all isolates, its use as appropriate target for detection of *Salmonella* may be in doubt. In view of the fact that this study was carried out with limited number of isolates, further studies could be carried out with more isolates further investigating the presence of *inv* A gene in all isolates and also its absolute suitability as the good and appropriate target for the molecular detection of *Salmonella.*

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