



Comparison Between Two Enrichment Broths for Detection of *Salmonella* from Reptilian Feces and Cloacal Swabs

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Abstract

Reptiles are shown to harbor and shed *Salmonella* without having any sign of illness and with notable rates that could pose a health hazard for their owners. In order to find a suitable enrichment broth for the recovery of *Salmonella* from these animals' feces Rappaport Vassiliadis (RV) broth was compared with selenite F (SF). Two sets of samples were collected from 270 reptiles in Tehran province which belonged to 46 reptilian species of 22 families. The specimens included 171 cloacal swabs, 20 feces, and 79 terrarium water specimens (in which the reptile defecates) according to their availability and reptile species. Each sample was separately enriched in RV and SF enrichment broths and then inoculated in three culture media to increase the possibility of *Salmonella* detection (CHROMagar *Salmonella*, MacConkey agar, and xylosine-lysin desoxycholate) and later checked for their biochemical properties as *Salmonella* spp. A total of 147 (54.4%) specimens were found positive for *Salmonella* using both enrichment broths. The positivity rate for RV (49.6%) was significantly higher than SF (40.4%) showing that RV could be considered a better enrichment broth for *Salmonella* recovery in feces or cloacal swabs ($p = 0.031$). In addition, the reptilian feces samples were more likely to have positive culture results than cloacal swabs and terrarium water in RV and SF broths ($p = 0.001$ and 0.000 , respectively); suggesting that, feces can be better specimens for detection of *Salmonella* intestinal shedding in reptiles or other animals.

Keywords: Enrichment Broth; Reptile; *Salmonella*

Introduction

Salmonellosis is an important public health problem that affects more animals and people compared to other single diseases [1,2]. It is a zoonotic disease and many animals act as reservoirs. Reptiles are shown to harbor *Salmonella* without having any sign of illness with a prevalence that could reach 90% [3]. More than 1300 serotypes have been reported in reptiles; from which very few were isolated from human or have been associated with reptile contact and zoonosis [4]. Cases of reptile-associated salmonellosis in human were not uncommon, some of them being fatal [5,6]. In response to those cases, the U.S. Food and Drug Administration (FDA) banned the importation of small turtles (carapace smaller

than four inches) which lead to a reduction in turtle associated salmonellosis [7].

These facts highlight the hazard that the *Salmonella* sp. Shed by reptiles represents for human health. It also underlines the importance of this pathogen's detection concerning health control programs [8]. Using an effective method for *Salmonella* detection in control programs, helps a more reliable infection rate estimation possible since using different culture methods for detection of *Salmonella* species has also been found to have a significant impact in the possibility of *Salmonella* detection [9]. When considering culture methods, selecting the appropriate enrichment broth

is very important; especially in the feces or cloacal swabs which needs a strong inhibition of the competitive bacteria while it allows the growth of *Salmonella* sp. [10,11]. Therefore, this study was designed to find out which one of the two enrichment broths (Rappaport Vassiliadis or Selenite F) works better for the detection of *Salmonella* species in the feces and cloacal swabs of reptiles.

Materials and Methods

Sampling

A total of 270 samples from reptiles of all ages referred to the specialized veterinary clinics in Tehran were studied. Three types of samples were collected: 1-cloacal swabs (two for each reptile), 2-feces (10g), and 3-terrarium water (10 ml). The choice of the sample type was related to the reptilian species and the availability of the specimen. In the case of terrarium water owners were asked to keep the water unchanged for at least five days before the sample was collected in order to increase the chance of *Salmonella* detection. In case of unavailability of feces (even after motivation) cloacal swab was obtained.

Salmonella isolation

All samples were divided into two parts and inoculated in two enrichment broths: Rappaport Vassiliadis broth (RV) and selenite F broth (SF) (Merck, Germany). Inoculated broths were then incubated for 12-18 h at 42°C and 37°C, respectively. Then both enrichment broths were inoculated and streaked for isolation on CHROMagar *Salmonella* (France), MacConkey agar (Merck), and xylosine-lysin desoxycholate (XLD) (Merck, Germany), and incubated in 37°C for 24 - 36h. Presumptive *Salmonella* colonies of each culture media were picked and subjected to biochemical tests with the use of standard media (Urea (Diffco, USA), Triple Sugar Iron (TSI) (Merck, Germany), peptone water (Difco, USA), Simmons' Citrate agar (Merck, Germany), and MRVP (Difco, USA) and procedures to make sure they belonged to the *Salmonella* spp.

Statistical analysis

Chi-square test was used to detect significant difference between the enrichment broths and positive *Salmonella* cultures. This test was also used to find out if the proportion of positive culture results in each broth was affected by the type of specimen used. In order to find out which type of specimen led to more positive culture results in each enrichment broth, multiple comparison with Bonferroni correction was conducted. The SPSS software (version

20; SPSS Inc. Chicago, IL, USA) was used for the analysis and a p value of less than 0.05 was considered statistically significant.

Results and Discussion

Samples culture results

Specimens including water (79 samples), feces (20 samples) and cloacal swabs (171 samples) were collected from 46 reptile species of 22 families. Of the total 270 reptiles from which samples were obtained, 82 were kept in humid, 86 in arid, and the rest, 102 were kept in semi-aquatic environments. Table 1 shows the positive and negative culture results of each enrichment broth. A total of 147 (54.4%) specimens were found positive for *Salmonella* using both enrichment broths. Table 2 gives the detailed results of *Salmonella* cultures according to the type of the specimen used in this study.

Table 1: Distribution of *Salmonella* culture results of 270 reptile samples in RV and SF broths.

Enrichment broth	Culture Results		Total samples
	Positive	Negative	
Rappaport Vassiliadis broth (RV)	134	136	270
Selenite F (SF)	109	161	270
Total*	147	123	270

*The overall culture results regardless of the broth type.

Table 2: Distribution of positive samples according to the specimen type in RV and SF broths.

Enrichment broth	Cloacal Swab	Specimen type			Total
		Water*	Feces		
RV	Positive Count	89	29	16	134
	% within Specimen type	52.0%	36.7%	80.0%	49.6%
SF	Positive Count	72	21	16	109
	% within Specimen type	42.1%	26.6%	80.0%	40.4%
Total	Count	171	79	20	270
	% within Specimen type	100.0%	100.0%	100.0%	100.0%

*Terrarium water is the water in which the reptiles defecate in.

Statistical analysis

The Chi-square test revealed that there was a strong difference between the proportion of positive *Salmonella* culture results in the two enrichment broths used in favor of the broth RV (p value =0.031). In addition, the estimated difference between the positive proportion of RV and SF was 0.092 With a 95% confidence interval (0.009, 0.176).

In addition, according to the Chi-square test the positive proportions of various types of specimens were found significantly different in each broth (p value = 0.001 and 0.000 in RV and SF, respectively). Using multiple comparison with Bonferroni correction showed that terrarium water samples were less likely to be positive than feces or cloacal swabs, while feces were the most likely to give positive culture results in both enrichment broths (Table 2).

Results of our study showed that more than half of the reptiles shed *Salmonella* in their feces (54.4%), with the use of two enrichment broths (Rappaport Vassiliadis and Selenite F) and three sample types (feces, cloacal swabs and terrarium water). Healthy reptiles have been shown to excrete *Salmonella* in their feces according to studies [12] and keeping reptile pets could be considered a menace to people who are immuno-compromised [13]; therefore, the considerable shedding rate in the reptiles of our study does not seem uncommon. Rappaport Vassiliadis broth was found superior to Selenite F for enrichment of reptile feces and cloacal swab samples for *Salmonella* isolation in our study (p = 0.031). This finding agrees with other researchers who reported the superiority of Rappaport Vassiliadis broth to other enrichment broths for recovery of *Salmonella* from poultry [14], meat products [15] as well as seawater that contains low or moderate pollution levels [16].

Our results also showed that the type of specimen used to detect *Salmonella* can possibly affect the positivity of the results in both tested enrichment broths; in that feces samples were more likely to be positive than other samples while terrarium water samples were less likely to be positive for *salmonella*. Feces contain more of the microorganisms shed by the intestines compared to cloacal swabs and the terrarium water (in which the reptile defecates in); thus, making our finding somehow reasonable. This finding also agrees with other researchers since a considerable difference in the positivity of cloacal swab samples obtained from a laying hen farm was reported compared to the feces samples [17]. Another study in layer breeder flocks also found similar results reporting

21.4% *Salmonella* positive rate in the wet feces compared to 8% in cloacal swabs [18]. Having said that, we should also mention that the uneven and variant distribution of our samples among the three groups of specimen type could have impacted the results and larger scale studies are warranted.

Conclusion

Our study showed that Rappaport Vassiliadis broth is a more reliable enrichment broth than Selenite F for detection of *Salmonella* in reptile feces and cloacal swabs. We also recommend that in case of assessment of intestinal shedding, feces samples be used whenever possible for *Salmonella* recovery in reptiles or other animals. The considerable rate of *Salmonella* shedding in the healthy reptiles' feces which was found in our study also warrants cautious care in the handling of young children or immunocompromised people to all reptilian species.

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Conflict of Interest

The authors have no conflict of interest to declare.

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