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Author(s)	SATO, Gihei
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# DETECTION OF SALMONELLA AND ARIZONA ORGANISMS FROM SOIL OF EMPTY TURKEY YARDS\*

Gihei SATO

*Department of Epizootiology  
Faculty of Veterinary Medicine  
Hokkaido University, Sapporo, Japan*

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In studies on the natural history of Salmonella or Arizona infections of poultry, it is essential to determine the role that contamination of the range plays in perpetuating the problem. Such knowledge is particularly important for an understanding of these infections in breeder turkeys, which are reared on many different kinds of ranges having numerous animal vectors of Salmonella and Arizona infections.

Specific studies on contamination of soil of turkey yards with Salmonella or Arizona organisms during and between breeding seasons are lacking, although a recent report<sup>2)</sup> showed the results of non isolation of Salmonella from ground soil of turkey houses and range soil. Accordingly, three ranches were selected for evaluation of contamination with the enteric organisms. One ranch had a history of Arizona infection in the hens the previous season.

This paper deals with the results of this investigation.

## MATERIALS AND METHODS

**Soil samples** Samples were taken from turkey yards which had not been used for 6 to 7 months since the previous breeding season. Samples of the top 2 cm of soil were put into circular half-pint cartons. All samples, except those from ranch C, were examined on the day of sampling or the next day. After the first examination, the samples were kept in a refrigerator for future use.

**Culture of soil samples** Two grams of each sample were inoculated into separate tubes containing 20 ml of selenite cystine broth each. The inoculated broth was subcultured onto brilliant green and bismuth sulfite agar plates. Five to ten Salmonella- or Arizona-like colonies, including rapid lactose-fermenting ones, were checked by serological and biochemical procedures. Serotypes of the isolates were identified at the State Public Health Laboratory, Berkeley, California.

**Count of viable organisms of Salmonella or Arizona in soil samples** Most

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\* This study was done at the School of Veterinary Medicine, University of California, Davis, California, U. S. A.

probable number (MPN) of viable organisms in the soil samples were calculated as follows: Ten grams of soil were diluted to 100 ml with sterile normal saline solution. A 10-ml sample of this original suspension was transferred into each of five tubes containing 50 ml of selenite cystine broth; 1.0- and 0.1-ml samples respectively were inoculated into each of five tubes containing 10 ml of the broth. A 100-fold dilution then was prepared from the original suspension. One ml (0.01 ml of the original suspension) of this dilution was inoculated into each of five broth tubes. Dilutions containing  $10^{-3}$  and  $10^{-4}$  ml of the original suspension were made. All tubes of broth were incubated overnight and subcultured on brilliant green agar the next day. The number of Salmonella- or Arizona-positive tubes was decided from the growth on the plates. Final calculation was made according to the table of MPN<sup>1</sup>).

### RESULTS

Isolation of Salmonella and Arizona organisms from soil samples Since the December samples from ranches A and B were taken in rainy weather, most of them were wet. Only soil from ranch A yielded Salmonella at a high frequency (tab.). Arizona was also isolated from one sample from ranch A.

TABLE *Isolation of Salmonella and Arizona organisms from soil samples from empty turkey yards*

RANCH	DATE OF SAMPLING	NO. OF PENS EXAMINED	NO. OF SAMPLES EXAMINED	NO. OF ISOLATIONS OF	
				Salmonella	Arizona
A	12-28-64	12	12	7 (58%)	0
A	2- 9-65	4*	20	0	1 (5%)
B	12-28-64	12	12	0	0
C	1-26-65	20	20	0	0

\* From each pen, 5 samples were taken.

In 1966, about one year later, 23 additional samples were taken at random from other ranches. The samples yielded neither Salmonella nor Arizona.

Relating the recovery of Salmonella and Arizona to the medium used, two of seven Salmonella-positive samples were identified only on brilliant green agar and other two only on bismuth sulfite medium. The remaining cultures were identified on both kinds of media. Isolation of Arizona was successful only on brilliant green agar plate.

Serotypes and viable count of the soil Four serotypes of Salmonella were detected from 7 samples. *S. livingstone* from 2 samples, *S. anatum* from 2, *S. binza* from 1 together with *S. livingstone*, and *S. worthington* from 3. Arizona 7:1,7,8 was isolated from one sample. Viable count of Salmonella or Arizona was examined in five samples which had been positive at the first examination. One of the samples gave Salmonella again (940 viables per gram). The remaining samples were negative.

Relation of soil contamination to the status of flocks To compare the Salmonella and Arizona status of ranches A and B with contaminated yard soil, cloacal swabs were

taken from 50 adult turkeys of each ranch in December, 1964. The birds were progeny of hens which had been kept in these yards the previous season. The cloacal samples were examined for Salmonella and Arizona organisms in a similar manner.

Three (6%) of the 50 birds from ranch B had *S. binza*. Cloacal samples from ranch A were negative, although that ranch had a history of Arizona 7:1,7,8 from dead embryos the previous season.

#### DISCUSSION

It is not clear whether the Salmonella and Arizona organisms detected from the soil in this study originated from infected birds in previous season or from carrier animals. However, Salmonella can survive for a long time in the soil, depending on such environmental conditions as nature of the soil, pH, humidity, contamination of the soil with other species of bacteria, and sunlight<sup>3</sup>. Possibly, isolation of Arizona organisms from the soil of ranch A may be due to contamination from breeders that had been on the range the previous season. It is difficult to explain why Salmonella was detected only on ranch A.

One sample contained 940 viable Salmonella per gram. This number is large when compared with fewer than 40 viable Salmonella organisms per gram found by YAMAMOTO et al. in the litter 44 days after turkeys were fed large numbers of *S. typhimurium*. These observations indicate that, under certain range conditions, Salmonella may survive easily or actual multiplication of the organisms may occur. In the study by YAMAMOTO et al., the pens had concrete floors which were open to sunlight. Some of the pens in this study were shaded by large oak trees. However, the temperature during the summer is sufficient for multiplication of the organisms.

#### SUMMARY

Sixty-four samples of soil taken from turkey yards of 3 ranches which had not been in use for 6 to 7 months were submitted for examination for Salmonella and Arizona groups. Seven (58%) of 12 samples from 12 pens of a ranch yielded *Salmonella livingstone*, *Salmonella anatum*, *Salmonella binza* and *Salmonella worthington*. One (5%) of 20 samples from other 4 pens of the same ranch had Arizona 7:1,7,8. One sample had 940 viable Salmonella per gram.

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