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Original Research Article

DETECTION OF *SALMONELLA* IN YOUNG CHICKS WITH CLOACAL SWABS

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ABSTRACT

Background: A non-destructive method such as a cloacal swab is very important to the poultry industry because it allows the breeder companies to determine if *Salmonella* is present in the valuable breeder chicks without the necessity of sacrificing any birds.

Objective: To evaluate a nondestructive procedure to detect the presence of *Salmonella* in valuable breeder chicks.

Methodology: Day-of-age broiler chicks from a commercial hatchery were orally gavaged with a marker strain of *Salmonella* Typhimurium (ST), and then placed in isolation units with drinkers, mesh flooring and feeders. At 7 and 14 days, 10 birds were cloacally swabbed (shallow and deep). Swabs were inserted 1 cm (shallow) into the cloaca and 2 cm deep (into the colon). Each swab was immersed in 5.0 ml of buffered peptone water (BPW), then streaked onto brilliant green sulfa agar plates with 200 ppm nalidixic acid (BGSN). Tubes and plates were incubated 24 h at 37^0C. When plates were negative, the pre-enriched tubes were vortexed and once again plated onto BGSN. The procedure for the frozen swabs was the same, except that 15% glycerol was added to the BPW and then frozen at -20C for 14 days. They were then thawed and analyzed as above. After swabbing, the chicks were sacrificed. The ceca was removed, placed in a stomacher bag, and macerated with a rubber mallet. Enumeration of the marker strain followed.

Results: When the level of the marker ST was greater than 10^6 CFU/g of ceca and cecal contents, detection with either shallow or deep swab was the same 94% (47/50). When the level of ST was less than 10^6 CFU/g, detection with either method fell to 63.3% (38/60). After 14 days freezing the swabs at -20C, the shallow method detected 50% (32/64), and the deep swab detected 64% (41/64).

Conclusion: Regardless of the level of ST in the ceca, there was no difference in the detection rate between the shallow or the deep swab. Even when using low inoculum levels, a high rate of ST colonization in the ceca was reached, allowing both the shallow and deep swabs to almost all be positive at 7 days of age. Since the poultry companies may not be able to analyze the swab results on the same day as collection, we decided to evaluate if freezing the swabs for as long as 14 days would have any adverse effect on the recovery of ST. Freezing did not seem to make a difference in sensitivity. For frozen swabs the deep

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colonal swab yielded better results than the shallow swab. So you can freeze if you must, but doing the analysis the same day as the swabbing (unfrozen) techniques seems to be slightly more reliable.

INTRODUCTION

Salmonella is one of the leading foodborne pathogens often found on poultry products. For the poultry industry, controlling and reducing the presence of this organism is a priority. Federal regulations are already in place using HACCP in order to reduce risks of *Salmonella* but in the future, meeting these standards will become even more important. Over the last decade the poultry industry has increased biosecurity at the farm level and introduced *Salmonella* vaccination programs in parents stocks [1, 2]. Even with these interventions, a portion of the health and safety risks of salmonellosis is still connected to raw poultry products. Many factors contribute to *Salmonella* in poultry, one of which is a newly hatched chick is susceptible to *Salmonella* colonization because its intestinal microflora is still immature [3]. Milner and Shaffer [4] found day old chicks could be readily colonized with very low doses of *Salmonella*. Another study by Cox et al. [3] demonstrated the same results. Chickens exposed to higher doses of *Salmonella* have a higher probability of becoming colonized with some *Salmonella* spp colonizing the avian intestinal tract more efficiently than others [5].

The objective of this research was to evaluate non-destructive methods to detect the presence of *Salmonella* in valuable breeder chicks. Many of the techniques used today, chick box liners, litter drag swabs, dust swabs, air samples and feed samples, do not directly evaluate how many individual birds are positive for *Salmonella* [1]. Cloacal swabs are a minimally invasive method which might provide reliable data that can be used to evaluate individual chick and flock *Salmonella* status. Previous research using cloacal swabs has been carried out on freshly slaughtered carcasses [6]. Intervention during live production, especially at the breeder flock level, is an important place to begin controlling the organism. Intervention at the breeder flock level could reduce the load of *Salmonella* being introduced to broiler farms, essentially reducing the amount of *Salmonella* present on live birds at the processing facilities. A non-destructive sampling method (shallow and/or deep cloacal swabbing) was evaluated. Since the poultry industry might not have the lab resources available for immediate evaluation of samples, freezing the swab samples was also evaluated.

MATERIALS AND METHODS

Newly hatched broiler chicks were obtained from a local hatchery and transferred within an hour to the University of Georgia Poultry Research Center. Twenty-five birds were orally gavaged with an inoculum ranging from 10^1 to 10^5 cells of a nalidixic acid resistant strain of *Salmonella* Typhimurium (ST). The inoculum, 0.5 mL volume, was gavaged directly into the crop using a 20 gauge animal feeding needle[3] (Popper and Sons, Inc., New Hyde Park, NY). After inoculation, birds were placed in isolation units (IU, Controlled Isolation Systems, Inc., San Diego, CA). The units contained nipple drinkers, mesh flooring and feeders for the chicks. Air exchange inside the IU was provided by a filtered positive pressure HEPA ventilation system. Chicks were fed non-medicated starter feed for the length of the experiment. Adequate brooding temperatures were maintained and birds had *ad libitum*

access to feed and water on a 24 hour light regimen.

Cloacal swabs

Unfrozen. At 7 and 14 days post challenge, 10 birds per treatment were subjected to a shallow, followed by a deep cloacal swab using two sterile polyester tipped applicators (Pur-Wraps, Puritan Medical Products Co, LLc, Guildford, ME). The shallow swab was inserted first at 1 cm in the cloaca followed by a deep swab inserted to a depth of 2 cm into the colon. Each swab was placed into separately labeled, 13 x l00 mm screw cap tubes containing 5.0 mL of 0.05% buffered peptone water (BPW). Excess length of the swab handle was cut with alcohol rinsed scissors. Samples were taken to the lab at Russell Research Center to be processed. Tubes were vortexed; streaked for isolation onto brilliant green sulfur agar (Difco/Becton, Dickinson and Co, Sparks, MD) with 200 ppm of nalidixic acid (Sigma Chemical Co., St. Louis, MO; BGS w/Nal) added; and the plates and the sample tubes were incubated 24 h at 37°C. Plates were observed for typical growth and, if present, recorded as positive; however, when the streaked plates were negative, the incubated sample tube was vortexed and streaked for isolation onto BGS w/Nal plates, incubated 24 h at 37°C, and final results were recorded.

Frozen. The same collection procedures used for the unfrozen shallow and deep cloacal swabs were used for the frozen samples from 7, 10, 14 and 21 day old chicks except that each swab was placed into 5 mL of 0.05% BPW plus 15% glycerol (Sigma, St. Louis, MO). The samples were stored frozen at -20°C for fourteen days, removed from frozen storage, thawed at ambient temperature for 4 h and processed for analysis according to the same sampling procedure as the unfrozen samples. The plates and tubes were incubated for 24 h at 37°C. Positive results were recorded; negative samples were struck onto BGS w/Nal agar plates following the same protocol used for the unfrozen samples, after which final results were recorded. Random colonies from both the frozen and unfrozen samples were checked to determine that they exhibited the same biochemical reactions and serogroup as the ST used for inoculation.

Ceca Sampling

After collection of the swab samples the broiler chicks were humanely euthanized and the exterior of chick was sprayed with 70% ETOH (Pharmco-Aaper, Brookfield, CT) to disinfect the surface. The skin, along with feathers, was separated and removed from the muscle wall. The muscle wall was sprayed with 70% ETOH, and an incision was made with an alcohol/flamed scalpel. The ceca were aseptically removed and placed into sterile stomacher bags (Fisher Scientific, Pittsburgh, PA). Bags were labeled accordingly, placed on ice and transported to the laboratory for analysis. The ceca were macerated using a rubber mallet to ensure the contents were exposed. BPW was added to the ceca at a ratio of 3:1 v/w before stomaching for 60 s (Technar Company, Cincinnati, OH). A semi-quantitative method was used to estimate the CFU/g in the ceca [7]. Briefly, two cotton tipped applicators were inserted into the bag containing the ceca/BPW solution. One swab was spread plated on BGS w/Nal plates (A plate). The second cotton tipped applicator was transferred to a tube containing 9.9 mL of BPW and vortexed for 10 s. A third cotton tipped applicator was inserted into this tube and spread plated onto a second BGS w/Nal plate (B plate). The contents of the tube were returned to the stomacher bag containing the ceca.

Plates and stomacher bags were incubated at 37°C for 24 h. When both spread plates were negative, a fourth cotton tipped applicator was inserted into the pre-enriched cecal samples and spread plated onto a fresh BGS w/NaI plate (C plate). The $\log_{10}$ value CFU/g of cecal material were calculated, recorded and log transformed.

RESULTS AND DISCUSSION

The results of a shallow and deep swab to detect *Salmonella* in young chicks 7 or 14 days of age are shown in Table 1. When the level of colonization in the ceca was > 10^6 CFU/gm, detection by either shallow or deep swab was 47/50 (94%). When ST colonization in the ceca was < 10^6 CFU/g, the detection rate by either shallow or deep swab was 38/60 (63.3%). However, regardless of the level of ST in the ceca, there was no difference in the detection rate between the shallow or the deep swab. Even when using lower inoculum levels, a high rate of ST colonization in the ceca was obtained after 7 days in the ceca of the chicks, allowing both shallow and deep swabs to be almost all positive at 7 d of age. Therefore, the shallow would be the better method to use because of less risk of injury to the chick. A non-destructive tool such as a cloacal swab is very important to the poultry industry to allow the breeder companies to determine if *Salmonella* is present in the valuable breeder chicks without the necessity to sacrifice any animals.

Table 1. Use of a shallow and deep cloacal swab to detect *Salmonella* in young chicks.

			Number Positive		
Age of Chick	Inoculum	# Sampled	Shallow	Deep	Level in ceca
7d	10^5	10	10	10	>10^6
	10^3	10	10	10	>10^6
	10^2	15	14	14	<10^6
	10^3	10	10	9	<10^6
	10^1	10	8	8	>10^6
14d	10^5	10	9	9	>10^6
	10^3	10	10	10	>10^6
	10^3	10	6	7	<10^6
	10^1	10	8	9	<10^6

Table 2. Effect of freezing for 14d on the shallow and deep swab to detect *Salmonella* in young chicks.

		Number of Positives		
Age of Chick	Inoculum	# Sampled	Shallow	Deep
7d	10^1	10	3	6
	10^3	10	5	8
10d	10^2	11	7	9
14d	10^1	9	2	3
	10^3	10	6	7
21d	10^2	14	9	8

Due to various constraints (such as sampling multiple flocks, distance between the farm and laboratory, etc.) the poultry industry may not be able to analyze the swab results on the

same day as collection. Therefore, we decided to evaluate if freezing the swabs for as long as 14 days would have any adverse effect on the recovery of ST. Table 2 shows the detection rates after freezing the cloacal swab samples from 7 to 21 day old chicks. These day-of-hatch chicks were orally inoculated with 10^1 to 10^3 ST cells per chick. After sampling and freezing for 14 days, the shallow swab detected 32/64 (50%) while the deep swab detected 41/64 (64%). Freezing did not seem to make a difference in sensitivity. If you are unable to analyze the swabs right away, and you must freeze them, it would seem that the deep colonal swab would give the best indication of *Salmonella* contamination.

The level in the ceca (for the frozen swab samples) was not determined, but the percent positive of the frozen swabs suggests that the levels were < 10^6 since similar results (50-64%) were obtained with unfrozen samples when the level of ST was < 10^6 (Table 1). Even after 14 days of freezing, using cloacal swabs gives a sufficient indication of *Salmonella* contamination in young chicks. So you can freeze if you must, but doing the analysis the same day as the swabbing (unfrozen) technique seems to be slightly more reliable.

Although several studies involving cloacal swabs have been published, this study is unique in several ways. To begin with, to be able to correlate a positive or negative swab result with the level of *Salmonella* in the ceca, you probably need to use a marker strain of *Salmonella* as we did in this study. There are no published studies that we could find that used a marker strain. Secondly we could find no other study in the literature that froze cloacal swabs prior to analyzing them. Cloacal swabs are a minimally invasive procedure which may be useful to the poultry industry. However one drawback is that each bird has to be handled individually to be sampled, and this may limit its usefulness.

CONCLUSION

In summary, regardless of the level of ST in the ceca, there was no difference in the detection rate between the shallow or the deep swab. Even when using low inoculum levels, a high rate of ST colonization in the ceca was reached, allowing both the shallow and deep swabs to almost all be positive at 7 days of age. Since the poultry companies may not be able to analyze the swab results on the same day as collection, we decided to evaluate if freezing the swabs for as long as 14 days would have any adverse effect on the recovery of ST. Freezing did not seem to make a difference in sensitivity. For frozen swabs the deep colonal swab yielded better results than the shallow swab. So you can freeze if you must but doing the analysis the same day as the swabbing (unfrozen) techniques seems to be slightly more reliable.

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