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Comparison of cotton swab versus alginate swab sampling method in the bacteriological examination of broiler chickens

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SUMMARY

Comparison of bacterial counts of poultry carcass skin by the cotton swab and alginate swab methods showed no differences between the two sampling methods in total counts and Enterobacteriaceae counts. Also no differences were found in Salmonella isolations.

INTRODUCTION

Many methods have been elaborated to assess the microbiological condition of processed beef, pork and poultry carcasses (Barnes, Impey & Parry, 1973; Patterson, 1972). When evaluating different methods not only the number of bacteria that can be recovered is of interest but also the reproducibility of the method (Fromm, 1959; Leistner & Szentkuti, 1970). The latter factor is of major importance as the actual number of bacteria recovered by the method can be evaluated in relative terms (Simonsen, 1971). Furthermore the method of sampling should be simple, thus allowing more samples to be examined within a given time, and at the same time flexible so that it can be used in laboratories with all modern equipment as well as in processing plants.

Finally it should be non-destructive. This is particularly important for quality control purposes where it would be convenient to return the examined chickens to the production area. Simonsen (1971) proposed that in this respect swabbing is the method of preference. A cotton-wool or alginate swab for removing bacteria from a known area of surface is perhaps the most widely used method for sampling surfaces. A brief description and evaluation of various modifications of this technique have been given by Patterson (1971). Swab sampling involves swabbing the carcass skin exposed through an opening in a sterile metal template, agitating the swab in an appropriate fluid and plating dilutions of the fluid for subsequent colony counts. Experimental results of swab sampling are described, among others, by Mallman, Dawson, Sultzer & Wright (1958), Seekins, Guenther & Walter (1958), Fromm (1959), Kotula (1966) and Avens & Miller (1970). Either cotton or alginate swabs can be used, but in theory the alginate swab is preferred since the swab is completely disolved during agitation in the fluid before plating, which is not the case with cotton swabs.

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The purpose of the following experiments was to investigate the difference in effectiveness of cotton swabs versus alginate swabs in enumerating bacteria on the skin of fresh broiler chickens.

MATERIALS AND METHODS

'Sani-swab' prefabricated cotton tipped applicators from Dunlop Export, Ohio were used, while the alginate swabs were hand made from Calgitex Calcium alginate bacteriological wool to approximately the same size and shape as the cotton swabs. The swabs were enclosed in separate test tubes and sterilized for 20 min. at 120° C.

For the main experiment the chickens used were cooled ready-to-cook broilers obtained randomly from different shops in the area. The broilers weighed approximately 1 kg. each. The swabs were first moistened in sterile wetting solution composed of 8·5 g. NaCl, 1 g. peptone (Oxoid) and 1 ml. Tween 80 per litre of water. The thigh skin breast skin, and side skin of the chicken were then individually sampled using a cotton swab for one side and an alginate swab for the other. Each sample consisted of a 9 cm.^{2} area delineated by a metal template. The area was swabbed 5 times in each of four directions in the same manner. The cotton swab tips were broken off into 10 ml. of sterile physiological saline solution with 0·1 % peptone (pps). Alginate swabs were placed in 10 ml. of Ringer calgon (Oxoid). All tubes were shaken vigorously for 60 sec. Serial dilutions were then made up using pps solution. The total counts were determined by the pour-plate method using Plate Count agar (Oxoid) incubated at 30° C. for 3 days. The Enterobacteriaceae were counted using the pour-overlay method and Violet Red Bile Glucose agar (Oxoid) incubated at 37° C. for 20–24 hr. Counts were expressed as colonies/cm.^{2}.

To show whether or not Ringer Calgon solution had an effect on the death rate of the bacteria, the solutions from the alginate swabs were plated as above but after different time intervals.

The removal of different species of bacteria from the swab into solution was demonstrated by the following experiment. A swab was moistened with wetting solution and then 0·1 ml. of a known concentration of a population of bacteria was pipetted into the swab. Cotton swabs were placed in separate test tubes of pps solution and alginate swabs were put into solutions of Ringer Calgon. Dilutions from swabs inoculated with Enterobacteriaceae species were plated out using Violet Red Bile Glucose agar as described above. Dilutions from swabs inoculated with Pseudomonadaceae species were plated out on Heart Infusion agar and incubated at 5° C. for 10 days. These results were compared with a control consisting of the same amount of bacteria with which the swabs were inoculated, directly plated out.

Another part of the investigations involved testing specifically for *Salmonella*. Broiler chickens were swab sampled in a poultry processing plant directly after spin-chilling. An area of approximately 25 cm.^{2} of the side skin was swabbed with an alginate swab while the opposite side was swabbed using a cotton swab. Different experiments were carried out on different days, each experiment consisting

Table 1. *Cotton swabs versus alginate swabs in poultry carcass skin sampling*

	No. of samples	Experiments in which higher counts were given by: Cotton swabs	Alginate swabs	Probability*
Enterobacteriaceae counts				
Thigh skin	33	22	11	0·08
Breast skin	34	20	14	0·39
Side skin	34	19	15	0·61
Total counts				
Thigh skin	36	21	15	0·20
Breast skin	38	23	15	0·13
Side skin	37	23	14	0·09

* Sign-test, two sided significance.

of 50 broilers. The isolation of *Salmonella* was carried out by a method based on the reference method of the International Standard ISO 3565, Meat and meat products, detection of salmonellae. For this purpose the swabs were put into individual test tubes containing 20 ml. of a pre-enrichment broth of buffered peptone water. The test tubes were incubated for 18 hr. at 37° C. Following this, 2 ml. of each solution were transferred to 20 ml. of Muller Kaufmann tetrathionate broth. After incubation of this enrichment broth for 24 hr. at 43° C. all tubes were streaked on Brilliant Green agar (Oxoid) and incubated for 18 hr. at 37° C. From plates with suspected colonies of *Salmonella*, one colony was transferred to a slant of Kligler Iron agar (Oxoid, CM33) and to a lysine (Oxoid, CM308) tube. If both were positive then serological typing was carried out by the National Institute of Public Health, Bilthoven, The Netherlands.

RESULTS

In examination of the broiler skin with cotton and alginate swabs it was found that there is no difference between the two swabs (Table 1). The sign test rather than the *t*-test was used as the broilers did not belong to the same population. Bacterial counts of different chickens varied over a wide range, which is shown in Fig. 1 and 2, as the chickens had been stored for various number of days. From these figures it becomes clear that there is no difference in bacterial counts between the parts of skin sampled. Using the χ^2 test (Fisher, 1954) the aggregate of the probabilities of the 6 individual probabilities given in Table 1 and has a value of 0·21. This indicates also no difference.

Ringer calgon was shown to have no effect on the death rate of bacteria; even after 5 hr. no bacterial destruction occurred. These results are in conformity with former investigations.

The results of the experiment in which the swabs were inoculated with 0·1 ml. of a known concentration of bacteria are given in Table 2. It is evident that, depending on the bacterial type, not all the bacteria deposited on the cotton swabs were counted.

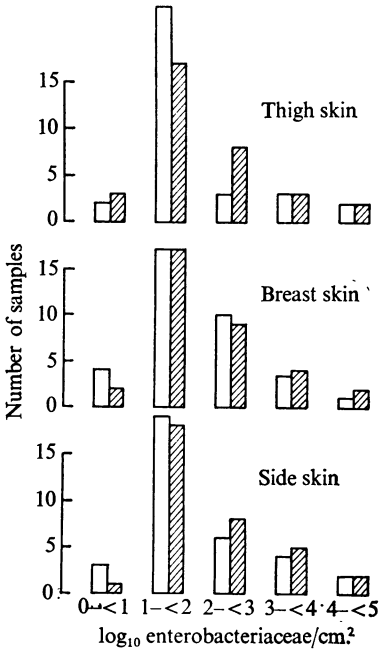

Fig. 1

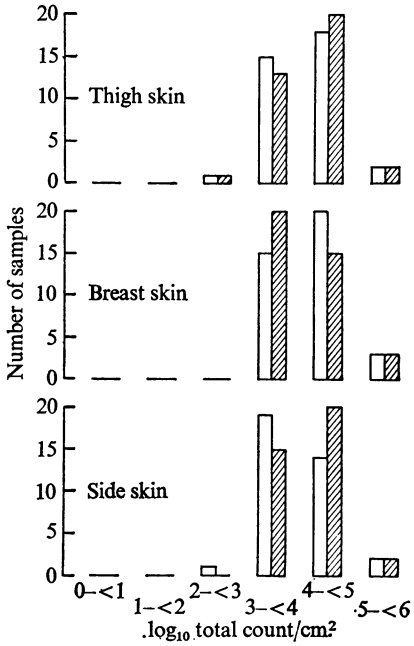

Fig. 2

Fig. 1. Enterobacteriaceae counts from chicken skin using alginate swabs □, and cotton swabs ▨.

Fig. 2. Total counts from chicken skin using alginate swabs □, and cotton swabs ▨.

Table 2. *Removal of different species of bacteria from the swab into solution*

	Control			Cotton			Alginate		
Bacteria species	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n
Salmonella oranienburg	2·06	0·04	12	1·73*	0·09	12	1·98	0·05	12
S. eastbourne	2·02	0·03	6	1·75*	0·14	6	1·92	0·07	6
S. typhimurium	1·98	0·06	5	1·71*	0·03	5	1·95	0·09	5
Escherichia coli K12	1·96	0·10	6	1·91	0·15	6	2·05	0·07	6
E. coli K12N97+	1·99	0·03	5	1·81	0·07	6	1·94	0·07	6
Citrobacter spp.	2·06	0·05	5	1·87	0·10	6	2·04	0·06	6
Klebsiella spp.	2·16	0·04	6	2·10	0·06	6	2·13	0·03	6
Pseudomonas fluorescens	1·99	0·09	6	1·71*	0·15	6	1·88	0·08	6
P. EBT/2/143	2·03	0·07	5	1·70*	0·17	5	1·89	0·03	5
P. putrefaciens	2·06	0·14	7	1·92	0·19	7	1·96	0·12	8

$\bar{x}$, $\log^{10}$ mean of number of experiments; s, standard deviation; n, number of samples.
* Significant difference at 95% level of confidence.

Table 3. *Results of salmonella investigation using cotton and alginate swabs*

Experiment number	Number of broilers investigated	C−, A−	C+, A+	C+, A−	C−, A+
1	50	30	2	9	9
2	50	39	2	4	5
3	50	47	0	3	0
4	50	47	0	2	1
5	50	44	2	3	1
6	50	32	4	8	6
7	50	45	0	2	3

C+, Cotton swab salmonella positive; C−, cotton swab salmonella negative; A+, alginate swab salmonella positive; A−, alginate swab salmonella negative.

In Table 3 the results of the salmonella investigation using cotton swabs and alginate swabs are given. The probability that cotton enumerates more *Salmonella* from the carcasses is 0·79 (sign-test, one sided significance). This means that there is no difference. From the salmonella serotypes isolated *S. agona* predominated (86 %) followed by *S. infantis* (9 %), *S. amager* and *S. livingstone* (each 2 %) and *S. orion* (1 %).

DISCUSSION

The object of this study was to compare the cotton swab and the alginate swab sampling method.

In the first part of the experiment it was shown (with 95 % confidence, two sided test) that there is no difference between cotton and alginate swabs in making total counts and Enterobacteriaceae counts.

The swab method requires two transfers of bacteria, one from the skin to the swab and one from the swab to the fluid. The first transfer has been shown to be incomplete (Fromm, 1959; Avens & Miller, 1970). From table 2 it is evident that fewer bacteria go into suspension from the cotton swab than from the alginate swab. From these results (Tables 1 and 2) it can be concluded that cotton swabs pick up a greater number of bacteria from the skin than alginate swabs do. This means that in quantitative determination of *Salmonella* on chicken skin the cotton swab might have advantages over the alginate swab. However, no difference was found (Table 3).

Swab sample bacterial counts may not approach the actual carcass bacterial count (Patterson, 1972; Avens & Miller, 1970). Also, the swab sampling method may not enumerate a consistent percentage of the actual carcass bacterial counts, depending on how firmly the bacterial flora is attached to the skin surface (Notermans & Kampelmacher, 1974, 1975*a*). The swab method appears to be particularly inadequate for counting bacteria on poultry carcass skin that has been subjected to a bactericidal treatment such as heat destruction (Avens & Miller, 1970; Notermans & Kampelmacher, 1975*b*).

If, despite the objections mentioned above, the swab sampling method is used then cotton as well as alginate swabs can be used without obtaining great differences in the results of the bacterial counts.

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