



Progress In Poultry

"THROUGH RESEARCH"

MICROBIOLOGICAL MONITORING OF HATCHERY AND HATCHING EGG SANITATION

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The centralization of the poultry industry into fewer and larger companies has resulted in an increase in the size and complexity of modern hatcheries. This change has been accompanied by improvements in hatchery equipment, design, and ventilation which have helped to make sanitation easier. At the same time the larger size of these units has reduced the direct supervision by management of many hatchery chores. Concurrently the goals and expectations of management for high hatchability and production of high quality chicks has increased. This situation has made it essential for hatcheries to practice routine microbiological monitoring of hatchery sanitation programs and egg sanitation.

This study was undertaken to assess the practicality and feasibility of using microbial monitoring procedures in commercial hatcheries.

Materials and Methods

The hatchery air and egg surfaces were sampled in fourteen hatcheries; nine were sampled twice. The technician traveled to the facility and dressed in clean overalls, a disposable head covering, and disinfected rubber boots before entering.

The air sampling technique recommended by Sadler (1975) was selected for use in this study due to its simplicity and low cost. Tryptic soy agar media plates were obtained from the University of California, College of Veterinary Medicine. They had been preincubated to assure sterility and were sealed in plastic bags. The plates were placed in the location to be sampled and the cover was removed and placed with open side down--next to the plate. After 10 minutes the technician returned and closed the plate. Plates were taped closed and returned to a clean plastic bag for transport. If visible dust was present on the area to be sampled, the plate was placed on a clean paper towel during the exposure. The areas sampled varied somewhat due to condition in the commercial facilities. Typical areas where air samples were taken included incubators, the incubator room, hatcher, the hatcher room, the egg storage room, the egg traying area, the chick pulling area, the chick vaccinating area, and the equipment washing area.

Hatching eggs were sampled using the technique described by Arhienbuwa et al., (1980). Eosin methylene blue agar plates were used to detect E. coli organisms. Sterile sampling

tape (Con-Tact-It) was obtained from Birko Chemical Corporation, P.O. Box 1315, Denver, Colorado 80201. This tape is enclosed in a plastic cartridge with a flat surface (16 x 18 mm) for consistent sampling. The tape was pulled from the Con-Tact-It cartridge over the special sample area on the cartridge and pressed on the media as a control. New tape was positioned and pressed on the large end of each egg in such a way that all of the tape surface made contact with the shell and the tape was then pressed on the EMB agar plate. Twenty-five randomly selected eggs were sampled from each of the 29 flocks checked.

All plates were returned to the University of California and incubated at 37°C until counts were made after 24 or 48 hours.

Results and Discussion

The results of air sampling in hatcheries are shown in Tables 1 and 2. Sadler (1975) has suggested standards for rating a hatchery's sanitary status using this air sampling technique. His standards are shown in Table 3 and are based on air sampling after cleaning or when areas should be clean. In our study, contaminated areas were intentionally sampled for contrast and as a positive control for the technique. These results are identified in the tables with an * to alert the reader that high counts are expected in these areas. Clearly some hatcheries rated good or excellent in all areas which were clean when sampled, while in others one or more areas were in need of attention. A definite build-up of molds was evident in Hatchery A (Table 1) on July 2.

In most cases the chick vaccinating area was the location where chicks were being processed, but in Hatchery E (Table 1) and Hatchery K (Table 2) on July 25, this plate was

exposed in the room where Marek's vaccine was mixed. In both cases the count was higher than expected.

After completion of this study, the authors concluded that the open plate air sampling technique can be a useful tool. If counts continue to be above expected levels, it would appear desirable to sample surfaces of air ducts or other possible reservoirs of contamination so that they can be cleaned to correct the problem.

The Con-Tact-It tape used on eggs in this study can also be used for surface sampling. Previous studies by Stinson and Tiwari (1978) have demonstrated that the tape can be used for a quick indication of surface contamination, but the counts will be considerably lower than would be found with swabbing or direct media contact techniques and are more variable. Therefore, it is recommended that at least two impressions be taken from each area to be checked to reduce sampling error.

Soucy et al. (1983) have used the Millipore Swab Test Kit to sample surfaces and reported that it was effective in locating contaminated areas in hatcheries. The most common reservoirs of organisms found were machine inlets, exhausts, and room floors. The materials for this procedure cost from \$3 to \$4 per sample, while similar costs for tape samples were \$.10 to \$.20 per sample.

Special media plates (Rodac plates) can be obtained from commercial sources and are designed for media contact sampling of surfaces. They are fast and easy to use. The cost is usually about \$.75 each. An adaptation of this technique using media slices was suggested by Shane (1975). It would reduce the cost of media contact sampling if a laboratory is available to prepare the media.

Table 1. Results of microbiological survey in broiler hatcheries.

Hatchery Code/Type	Date Sampled (mo/day)	Time of Plate Count (hrs)	Location Sampled											
			Egg Storage	Egg Tray Room	Incubator Room	Incubator	Hatcher Room	Hatcher No. 1	Hatcher No. 2	Chick Pull-Processing	Chick Vaccinating	Chick Holding	Equipment Washing Area	
			#bacterial colonies/#mold colonies											
A/Broiler (Big J)	5/21	24	1/0	2/0	16/1	29/0	NA ^a	TNTC ^{*b}	1/0	NA	NA	NA	NA	3/0
		48	4/1	5/0	26/1	32/0	NA	TNTC [*]	7/0	NA	NA	NA	NA	7/0
	7/2	24	2/31	16/12	16/32	0/7	0/1	19/0	NA	31/1	46/2 [*]	TNTC [*]	55/19	
		48	6/52	28/19	28/49	0/13	2/6	27/0	NA	43/11	68/6 [*]	TNTC [*]	63/27	
B/Broiler (Robbins)	5/21	24	8/0	16/12	2/0	NA	35/0	TNTC [*]	TNTC [*]	53/0 [*]	NA	NA	NA	
		48	20/0	28/19	2/1	NA	>35/6	TNTC [*]	TNTC [*]	>53/1 [*]	NA	NA	NA	
	7/2	24	0/0	1/0	3/0	0/0	24/0	128/3 [*]	NA	TNTC/4 [*]	48/3 [*]	53/3 [*]	NA	
		48	1/0	4/0	7/0	0/0	33/0	>128/8 [*]	NA	TNTC/8 [*]	63/9 [*]	71/7 [*]	NA	
C/Broiler (Big J)	5/21	24	38/0	85/0	84/0	NA	102/0 [*]	33/0	NA	153/0 [*]	83/0 [*]	118/0 [*]	NA	
		48	53/3	>85/3	>84/0	NA	102/6 [*]	>33/5	NA	>153/0 [*]	>83/0 [*]	>118/1 [*]	NA	
	7/2	24	5/0	78/0	10/0	NA	16/27	84/0 [*]	NA	TNTC [*]	98/1 [*]	93/0 [*]	NA	
		48	8/0	86/0	14/0	NA	26/32	98/0 [*]	NA	TNTC [*]	TNTC/4 [*]	TNTC [*]	NA	
D/Broiler (Robbins)	5/29	24	3/1	2/0	15/0	4/1	85/0 [*]	1/0	NA	TNTC/1 [*]	NA	NA	17/26	
		48	8/4	2/0	24/0	7/1	>85/0 [*]	1/1	NA	TNTC/1 [*]	NA	NA	17/42	
	7/24	24	0/0	12/0	10/0	1/1	80/0 [*]	6/1	NA	TNTC [*]	NA	NA	53/0	
		48	3/0	24/1	21/0	1/1	>80/5	6/5	NA	TNTC [*]	NA	NA	90/8	
E/Broiler (Big J)	6/12	24	NA	25/0	3/0	40/0	7/0	5/0	61/1 [*]	81/0 [*]	11/0	48/0 [*]	40/0	
		48	NA	30/0	3/0	>40/0	10/0	8/0	61/1 [*]	>100/0 [*]	16/0	70/0 [*]	46/0	
F/Broiler (Big J)	7/18	24	0/0	4/0	4/0	3/0	11/0	2/0	NA	137/0 [*]	NA	NA	6/1	
		48	0/0	10/0	5/9	5/34	18/1	3/0	NA	TNTC/2 [*]	NA	NA	8/3	
	8/28	24	0/0	0/0	29/1	3/2	127/1 [*]	10/1	NA	NA	NA	>300/0	42/>60	
		48	0/1	1/0	29/53	3/9	TNTC/11 [*]	10/12	NA	NA	NA	354/4	TNTC	

* Chicks present or sampled before cleaning.

^aNot available or not sampled.

^bTNTC = too numerous to count.

Table 2. Results of microbiological survey in Leghorn, turkey and duck hatcheries.

Hatchery Code/Type	Date Sampled (mo/day)	Time of Plate Count (hrs)	Location Sampled										Equipment Washing Area
			Egg Storage	Egg Tray Room	Incubator Room	Incubator	Hatcher Room	Hatcher No. 1	Hatcher No. 2	Chick Pull-Processing	Chick Vaccinating	Chick Holding	
#bacterial colonies/#mold colonies													
G/Leghorn (Robbins)	5/29	24	5/2	10/1	4/0	3/0	21/1	95/0*	138/0*	81/0	144/0	NA ^a	NA
		48	9/2	10/1	8/0	3/0	31/1	>95/0*	>138/0*	>81/0	>144/0	NA	NA
	7/24	24	0/0	3/0	2/0	1/0	3/0	0/0	NA	1/0	0/0	NA	NA
		48	0/0	4/0	7/0	1/0	3/0		NA	1/0	0/0	NA	NA
H/Leghorn (Buckeye)	6/11*	24	78/0	NA	8/0	0/0	12/0	46/0	26/0	73/0	71/0	TNTC ^b	NA
		48	TNTC	NA	TNTC	0/0	12/0	49/0	36/0	73/0	71/0	TNTC	NA
	7/25	24	9/0	NA	4/0	1/0	TNTC	TNTC	7/0	NA	NA	31/0	NA
		48	18/0	NA	10/1	3/0	TNTC	TNTC	11/0	NA	NA	51/0	NA
I/Leghorn (Robbins)	6/12	24	0/0	6/0	17/0	0/0	1/0	0/0	NA	3/0	NA	NA	9/0
		48	0/0	10/0	17/0	0/0	2/0	0/0	NA	11/0	NA	NA	14/0
J/Leghorn (Robbins)	6/12*	24	1/0	5/0	3/1	0/0	6/0	1/0	NA	TNTC	NA	NA	22/0
		48	3/0	7/0	6/1	0/0	8/0	4/0	NA	TNTC	NA	NA	30/0
K/Leghorn (Big J)	6/19*	24	39/0	NA	5/0	4/0	3/0	NA	NA	TNTC*	TNTC*	NA	19/2
		48	39/0	NA	5/0	4/0	4/0	NA	NA	TNTC*	TNTC*	NA	19/2
	7/25	24	9/0	0/0	6/0	0/0	2/0	0/0	NA	3/0	22/0	1/0	5/0
		48	11/0	1/0	7/0	2/0	3/0	0/0	NA	7/0	28/1	5/1	12/0
L/Turkey (Buckeye)	5/23	24	0/0	1/0	10/0	2/0	31/0	TNTC*	TNTC*	3/0	NA	NA	NA
		48	0/0	3/0	17/0	26/0	31/0	TNTC*	TNTC*	9/0	NA	NA	NA
	7/19*	24	2/0	10/0	2/0	0/0	TNTC*	TNTC*	NA	TNTC*	TNTC*	TNTC*	NA
		48	3/0	14/0	3/0	0/0	TNTC*	TNTC*	NA	TNTC*	TNTC*	TNTC*	NA
M/Duck (Robbins)	7/24	24	15/0	28/0	20/0	0/0	123/0*	0/0	NA	NA	NA	NA	NA
		48	20/0	52/0	34/0	5/0	TNTC*	0/0	NA	NA	NA	NA	NA

*Chicks, poults or ducklings present, or sampled before cleaning.

^aNot available or not sampled.

^bTNTC = too numerous to count.

Table 3. Standards for evaluating air sample counts from hatcheries (from Sadler, 1975)

Score	Counts		
	Setters	Rooms	Mold Contaminants
Excellent	0 - 10	0 - 15	0
Good	11 - 25	16 - 36	1 - 3
Average	26 - 46	37 - 57	4 - 6
Poor	47 - 66	58 - 76	7 - 10
Worse than poor	67 - 86	77 - 96	10 - 12
Miserable	87 & over	97 & over	13 & over

Egg Sampling

The results of tape sampling egg shells (Table 4) were more variable and difficult to read than had been hoped. Often E. coli were difficult to identify on the media due to extensive growth of other organisms. Additional investigation under field conditions would be necessary before a method of monitoring egg shell contamination could be recommended.

Despite the limitations of the method used, it was obvious that eggs washed or fumigated at the farm had lower E. coli counts than eggs fumigated at the hatchery or not treated. The large variability in E. coli counts from untreated egg samples may have been related to the length of storage before sampling which was known to vary in this study. Previous studies by Gentry and Quarles (1982) and by Furuta and Maruyama (1981) have shown that bacterial counts on egg shells

decrease when eggs are held in refrigerated storage and during incubation until pipping starts.

Summary

Open plate air sampling was found to be an easy and economical method of monitoring hatchery sanitation. The plate exposure and subsequent reading of plates required about two hours per hatchery. The material cost was about \$15 for 30 media plates.

The results of the egg sampling technique using sterile sampling tape were quite variable, although eggs washed or fumigated clearly had lower E. coli counts. The E. coli were often difficult to count on the eosin methylene blue agar plates due to extensive growth of mold and other bacteria. Additional testing would be required before a technique for egg shell monitoring under field conditions could be recommended.

Table 4. Results of hatching egg survey for E. coli.

Egg Type	Flock Number	Egg Treatment	Number Eggs <u>E. coli</u> Positive	Number <u>E. coli</u> Colonies
Broiler	1	Fumigated at hatchery	14	46
	2	"	5	7
	3	"	5	12
	4	"	0	0
	5	"	7	46
	6	"	1	3
	7	"	0	0
	8	"	20	132
	9	"	20	220
	10	"	7	15
	11	"	9	23
	12	No treatment	5	32
	13	"	5	18
	14	"	5	15
	15	"	22	TNTC ^a
	16	"	21	TNTC
	17	"	6	14
	18	"	3	3
Leghorn	1	Fumigated at ranch	1	3
	2	"	0	0
	3	No treatment	3	4
	4	"	1	1
	5	Unknown	5	12
	6	Fumigated at ranch	0	0
	7	"	0	0
	8	"	0	0
Turkey	1	Washed-Quaternary Ammonia	0	0
Duck	1	No treatment	22	71
	2	"	8	18

*.25-egg sample.

^a TNTC - Too numerous to count.

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