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A New Approach to Evaluate the Hygienic Condition of Commercial Hatcheries

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Abstract: Hatchery hygiene was evaluated in two commercial broiler hatcheries using open-plate method, surface swabbing and microbiological examination of hatchery fluff. Air samples and surface swabs were collected from: inside the hatch unit, the corridor outside the hatch unit and the chick processing room, while, fluff samples were collected after hatching from the hatch unit and the chick processing room. After cleaning and disinfection process of the hatch, four commercial disinfectants were evaluated for their effectiveness for controlling the hatchery contaminants, TH4®(combination of glutaraldehyde + quaternary ammonium compound), Virucidal extra® (chlorine preparation), Advantage 256® (phenolics) and Perasan® (per-acetic acid). The obtained results indicated that surface swabbing and microbiological examination of hatch fluff could detect higher degree of contamination than open-plate method in the two investigated hatcheries. Per-acetic acid preparations and (glutaraldehyde + quaternary ammonium compound) could reduce completely hatchery contaminants after 30 min of application. Conclusively, open-plate method is easy to perform and inexpensive but may give false indication that the air is clean when it is not. So, surface swabbing and microbiological examination of hatch fluff are more reliable methods for evaluating the hygienic status of a hatchery. Moreover, Surface swabbing method is more accurate than open-plate method in evaluating the decontamination process of the hatch. Hatchery sanitation and the proper use of effective disinfectant are essential for successful operation of any commercial poultry hatchery. Per-acetic acid and (glutaraldehyde + quaternary ammonium compound) proved their efficiency in controlling hatchery contaminants and can be used as safe alternatives to formalin in poultry hatcheries.

Key words: Hatchery hygiene, open-plate methods, fluff, TH4, perasan

INTRODUCTION

Hatchery hygiene is recognized as an important factor in healthy poultry production (Rodgers *et al*., 2003). Poor standards of hatchery hygiene may lead ultimately to an explosion of pathogenic organisms resulting in sever economic loss. The environment of a poultry hatchery is very susceptible to contamination by microorganisms which can adversely affect hatchability of the eggs and can result in embryonic deaths. Typical microorganisms which are believed to adversely affect chick quality and cause embryonic deaths include *E. coli, Staphylococci species, Streptococci species and Aspergillus fumigatus* (Sheldon and Brake, 1991).

Therefore, the development and maintenance of an effective hatchery sanitation program is essential for the successful operation of a poultry hatchery. Investigations have revealed large microbial populations in many hatcheries despite the application of various sanitation measures. The degree of contamination was first measured numerically by the microbiological examination of hatcher fluff, a method developed by (Nichols *et al*., 1967; Furuta and Maruyama, 1981; Chen *et al*., 2002) and later by the air sampling technique which is used extensively in the poultry industry to monitor bacterial and fungal levels in air and to evaluate the efficiency of decontamination (Chute and Gershman, 1961; Gentry *et al*., 1962; Ernst, 1987; Rodgers *et al*., 2003; Moubarak, 2007). Although these tests reveal the magnitude of the contamination in the hatching environment, they do not indicate where the organisms come from, how they reach the hatchers or where they multiply. This information can only be gained by periodically surveying the microbial populations of the many objects and surfaces which may harbor organisms in the hatchery.

Hatchery sanitation programs should include the use of one or more disinfectant to inhibit the growth of microorganisms and maintain a desirable level of hatchability of fertile hatchery eggs. Traditionally, formaldehyde has been utilized as the fumigant or disinfectant in many hatcheries in order to control the unwanted spread of microorganisms. However, other moderately effective disinfectants such as quaternary ammonium compounds, peroxides, glutaraldehyde and phenolics are also currently utilized in the poultry industry.

In normal use, formaldehyde gas is generated and released in hatchers by mixing formalin and potassium permanganate in specific ratios. This technique requires the handling of potentially hazardous chemicals by hatchery workers and possible exposure of the workers to the gas when initiating the chemical reaction. The use

of formaldehyde as disinfectant has further disadvantage, that formaldehyde has been suspected of being carcinogenic and hence faces possible further governmental regulation of its use (Sheldon and Brake, 1991). A need therefore exists for safe and effective disinfectants for use in hatchery sanitation programs which have the ability to inhibit the growth of microorganisms and maintain an acceptable level of hatchability of the eggs treated therewith. A need also exists for a disinfectant that is convenient to use and can minimize the time required for satisfactory sanitization. So, the objectives of the present study are:

- To evaluate the sanitary condition of two commercial broiler hatcheries using, open-plate method, surfaces swab and microbiological examination of fluff.
- To investigate the bactericidal and fungicidal efficiency of some available disinfectants other than formaldehyde in a trial to evaluate their effect in controlling contaminants of commercial hatcheries.

MATERIALS AND METHODS

The experimental work was carried out in two commercial broiler hatcheries located in Giza governorate. Samples were collected on four separate dates, as hatching chicks were being processed and after cleaning and disinfection process of the hatchery had been completed. On each visit, air samples and surface swabbing were collected from: inside the hatch unit, the corridor outside the hatch unit and the chick processing room, while, fluff samples were collected after hatching from the hatch chamber and from the chick processing room.

Open-plate method: At each sampling site, sterile *Petri*-dishes containing either plate count agar (for total bacterial count), Sabaurod's dextrose agar (for total fungal counts) or, MaConkey's agar (for total coliform count) were placed uncovered for 10 min at a height of one meter from the floor surface (Berrang *et al*., 1995).

Surfaces swab: Sterile moistened swabs with sterile normal saline were used to swab walls and floors of each sampling site. Swabs were received in sterile test tubes containing sterile normal saline and then, were transferred to the laboratory in ice box where 0.1 ml of each sample was plated on sterile plates of plate count, MaConkey's and Sabaurod's dextrose agar (Willinghan *et al*., 1996).

Fluff testing: After hatching, fluff was collected from the surfaces of racks, hatch baskets; corners of hatches and chick processing room then, samples were placed in clean sealed plastic sampling bags and were carried back to the laboratory in ice box. Under aseptic conditions 0.5 gram of the fluff was placed into 50 ml of sterile normal saline, mixed well, then 0.1 ml was inoculated onto each of sterile plates of plate count agar, MaConkey's agar and Sabaurod's dextrose agar (Chen *et al*., 2002).

All the inoculated plates and air sampling plates of plate count agar and MaConkey's agar were incubated at 37°C for 24-48 h while those of Sabaurod's dextrose agar were incubated at 25°C for 3-5 days and were then enumerated. Microbial levels were expressed as Colony Forming Units (CFU) per 10 cm diameter plate. Isolation and identification of the suspected colonies was done according to MacFaddin (1980) .Results are recorded in Tables (2-6).

Disinfection of the inner chamber of the hatchery machine: The inner chamber of the hatch machine was sprayed with one of four commercial disinfectants using the concentrations recommended by the manufacturers as shown in (Table 1). After 30 min of each treatment, aerial microorganisms were tested using open-plate method. Also walls and floors were swabbed as explained to judge the effectiveness of the disinfection process before.

RESULTS AND DISCUSSION

Hatchery sanitation was evaluated in two commercial broiler hatcheries designated as hatchery I and II, using

Table 1: The used chemical disinfectants and their concentrations

Disinfectant	Supplier	Composition		Used dilutions
TH4+®	SOGEVAL (France)	Glutaraldehyde	6.25%	0.5%
		Quaternary ammonium compound	12.5%	
		Terpine derivatives	4.0%	
Virucidal extra®	AVS Ltd, Northern	Potassium monopersulphate	23.0%	1%
	Ireland UK	Sodium dichloro-s-triazinetrion	5.0%	
Advantage 256®	Preserve international	Ortho-phenylphenol	11.0%	0.4%
	USA	Orth-Benzyl-para-chlorophenol	6.0%	
		Para-Tertiary-Amylphemol	4.0%	
		Inert engredient	79.0%	
Perasan®	Henkel (Germany)	Peracetic acid	5.0%	1%
		H_2O_2	20.0%	
		Acetic acid	10.0%	

Table 2: Total bacterial count, fungal and coliform counts recorded using open-plate method, surface swab and fluff testing methods

		Open- plate method			Surface swab method			Fluff testing		
Sampling site	Hatchery	T.B.C	T.F.C	T.C.C	T.B.C	T.F.C	T.C.C	T.B.C	T.F.C	T.C.C
Inside hatch unit	I	75	14	45	$50x10^2$	40x10	$30x10^2$	$80x10^2$	70x10	$30x10^2$
	II	60	10	30	$35x10^2$	20x10	$20x10^2$	$60x10^2$	55x10	22x10
Corridor outside hatch unit	I	30	8	10	$20x10^2$	25x10	$10x10^2$			
	II	22	5	3	$10x10^2$	10x10	60x10			
Chick processing room	I	128	17	35	$65x10^2$	$10x10^2$	$20x10^2$	$80x10^2$	$13x10^2$	$28x10^2$
	II	90	14	35	$50x10^2$	80x10	$18x10^2$	$68x10^2$	90x10	$23x10^2$

Table 3: Incidence of bacterial isolates recovered from the investigated hatcheries using open- plate method, surface swabs and fluff testing

			Salmonella sp.		*Pseudomonas sp.*		*Shigella sp.*		*Kelbesilla sp.*		*E. coli*	
Methods of sampling	Hatchery	No. of samples	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Open- plate	I	40	Nil	Nil	4	10	Nil	Nil	2	5	Nil	Nil
	II	40	Nil	Nil	3	7.5	Nil	Nil	1	2.5	Nil	Nil
surface swabs	I	64	4	6.25	5	7.81	5	7.81	3	4.68	4	6.25
	II	64	2	3.12	4	6.25	3	4.68	1	1.56	2	3.12
fluff testing	I	16	2	12.5	1	6.25	1	6.25	2	12.5	3	18.75
	II	16	1	6.25	1	6.25	Nil	Nil	1	6.25	2	12.5

			Enterobacter		*Yersiniae*		*Hafniae*		*Serratiae*		*Proteus*		*Gr+cocci*	
Methods of sampling	Hatchery	No. of samples	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Open- plate	I	40	1	2.5	2	5	1	2.5	3	7.5	5	12.5	4	10
	II	40	Nil	Nil	1	2.5	Nil	Nil	2	5	3	7.5	3	7.5
surface swabs	I	64	5	7.81	8	12.5	5	7.81	3	4.68	8	12.5	7	10.93
	II	64	3	4.68	4	6.25	3	4.68	1	1.56	5	7.81	2	7.81
fluff testing	I	16	3	18.75	3	18.75	2	12.5	2	12.5	3	18.75	3	18.75
	II	16	3	18.75	3	18.75	1	6.25	1	6.25	2	12.5	2	12.5

Table 4: Incidence of fungal isolates recovered from the investigated hatcheries using, open- plate, surface swabs and fluff testing method

			Moulds										Yeasts			
			A. fumigatus		*A. niger*		*A. flavus*		*A. terreus*		*Penicillium*		*Candida albicans*		*Rhodotorula sp.*	
Methods of sampling	Hatchery	No. of samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Open-plate	I	40	9	22.50	8	20.00	8	20.00	1	2.5	7	17.5	4	10.00	1	2.5
	II	40	5	12.50	4	10.00	4	10.00	Nil	Nil	3	7.5	2	5.00	Nil	Nil
Surface swabs	I	64	15	23.43	11	17.18	14	21.87	6	9.37	6	9.37	7	10.93	3	4.68
	II	64	8	12.50	7	10.93	5	7.94	2	3.12	2	3.12	2	3.12	1	1.56
Fluff testing	I	32	9	28.12	5	15.62	6	18.75	3	9.37	3	9.37	2	6.25	1	3.12
	II	32	5	15.62	3	9.37	3	9.37	2	6.25	1	3.12	1	3.12	1	3.12

open plate method, surface swabbing and microbiological examination of fluff. Results of total bacterial, fungal and coli form count are shown in Table 2. The obtained results indicated that surfaces swabbing and microbiological examination of fluff could detect higher degree of microbial contamination than open-plate method in the two investigated hatcheries. This finding is in agreement with that of (Kung'u, 2007) who stated that open-plate method is easy to conduct and of low cost, but it can only detect the viable micro-organisms; it may give false impression that the air is clean if most of the air born micro-organisms are dead. He added that false negatives may be in buildings with very restricted mold growths and very still air. Microbial contamination of the chick processing room in the two hatcheries was higher than the other sampling sites, a finding which coincides with those of (Shane, 1993; Sander and Wilson, 1999; Rodgers *et al*., 2003; Moubarak, 2007).

The observation that air-born bacterial counts were proportional to those of surface swabs suggesting that a direct relationship existed between them. The hypothesis that bacteria on horizontal surfaces may become air-borne from employee activity and could be drawn into the hatchers where they multiplied rapidly during hatching. As the chicks dried off, the organisms on fluff and dust spread through the rooms where they again settled and this cycle could be repeated with each hatch (Magwood and Marr, 1964; Davies and Wray, 1994).

The relative occurrence of various bacterial and fungal isolates recovered from the two hatcheries (Table 3 and 4) revealed that some bacterial strains could not be isolated through open-plate method although they can be detected by surface swab and microbiological examination of fluff. The obtained results are in accordance with those of (Chen *et al*., 2002) who proved that detecting micro-organisms in fluff is a convenient

Table 5: Effect of different disinfectants on the T.B.C., T.C.C and T.F.C of the hatch using surface swabbing

The used disinfectants	T.B.C			T.C.C			T.F.C		
	Before	After	Reduction %	Before	After	Reduction %	Before	After	Reduction %
TH4®	$50x10^2$	Nil	100.0	$30x10^2$	Nil	100.0	$4x10^2$	Nil	100.0
Virucidal extra®		Nil	100.0		Nil	100.0		5x10	87.5
Advantage 256®		$1x10^2$	98.0		1x50	98.3		$1x10^2$	75.0
Perasan®		Nil	100.0		Nil	100.0		Nil	100.0

T.B.C. = Total Bacterial Count T.C.C. = Total Coliform Count T.F.C. = Total Fungal Count

Table 6: Effect of different disinfectants on the T.B.C., T.C.C and T.F.C. of the hatch using open- plate method

The used disinfectants	T.B.C			T.C.C			T.F.C		
	Before	After	Reduction %	Before	After	Reduction %	Before	After	Reduction %
TH4®	75	Nil	100.0	45	Nil	100.0	14	Nil	100.0
Virucidal extra®		Nil	100.0		Nil	100.0		2	85.7
Advantage 256®		Nil	100.0		Nil	100.0		3	78.5
Perasan®		Nil	100.0		Nil	100.0		Nil	100.0

T.B.C. = Total Bacterial Count T.C.C. = Total Coliform Count T.F.C. = Total Fungal Count

method for evaluating the hygienic status in a hatchery. However, Berrang *et al.* (1995) found that swab samples may be negative for salmonella while hatching air samples may not be negative so, he concluded that environmental samples do not necessarily reflect the contamination in the air. From the recorded results in Tables 2-4, it could be noticed that the sanitary condition of hatchery II is better than that of hatchery I. According to literature this should result in lower degree of contamination of hatching eggs and consequently enhance the hatchability and improve the chick quality.

Effect of different disinfectants on T.B.C&T.C.C and T.F.C are shown in Tables 5-6. Both TH4+® (glutaraldehyde + quaternary ammonium compound) and perasan® (per acetic-acid) preparations recorded satisfactory results in controlling hatchery contaminant. The obtained results are agreeable with those of Suweify, 1999 who found that TH4+® was effective against *S. pullorum*, *E. coli* , *St. aureus, A. fumigatus* and *Candida albicans* in 15 min (McDonnell and Russell, 2001) who found that glutaraldehyde, has a broad spectrum activity against bacteria and their spores, fungi and viruses and Deeba *et al.* (2003) who proved that *S. pullorum*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Streptococcus faecalis* were 100% susceptible for TH4+® and Soliman *et al.* (2009) who proved that TH4+® is the most powerful disinfectants because of the synergistic action of the quaternary ammonium and glutaraldehyde bases.

However, Rodgers *et al.* (2001), Kaskova *et al.* (2007), Bauermeister *et al.* (2008) proved that per-acetic acid is an effective antibacterial agent in hatcheries, poultry houses and poultry processing plants. Virucidal extra® (chlorine preparation) could reduce the total bacterial and coliform count completely but can not reduce the total fungal count. These results are comparable to those of (Ramesh *et al.*, 2002) who found that chlorine based disinfectant was effective in controlling bacterial contaminants. However, advantage 256® (phenol) could not reduce both the total bacterial and fungal count after such contact time (Table 5); may be it needs a longer time or a higher concentration. This result is agreeable with those of (Sander *et al.*, 2002) who recorded variable degrees of bacterial resistance to advantage 256®. Also, from (Table 5 and 6) it can be noticed that, surfaces swabbing is more accurate than open-plate method in evaluating the decontamination process of the hatcher.

Conclusion: In conclusion, air sampling to monitor hatchery sanitation is an easy method to perform and inexpensive but it may give a false impression that the air is clean when it is not. So, surface swabbing and microbiological examination of fluff are more reliable methods for evaluating the hygienic status of a hatchery. Fluff from chicks can be send by mail to the laboratory for evaluation of the hygienic status in case of a distant hatchery. Otherwise, open-plate can be taken as a useful tool but, if counts continue to be above the expected levels, it would be necessary to sample surfaces. On the other hand, many available disinfectants in the Egyptian market as combination of per-acetic acid and (glutaraldehyde + quaternary ammonium compound) and are effective in controlling hatchery contaminations and safe than formaldehyde.

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