Research Article

HATCHERY HYGIENE MAPPING BASED ON MICROBIAL LOAD ASSESSMENT IN CHITWAN, NEPAL

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ABSTRACT

A study from March to August, 2016 was conducted to understand the level of hatchery hygiene in Chitwan using questionnaire survey and microbial load assessment. Key points of contact during chicks production were selected and contact plate samples (n=220) were collected aseptically from ten representative commercial hatcheries. Samples were inoculated and incubated in Nutrient Agar and Mac Conkey Agar for 24 hrs and in Sabouraud Dextrose Agar for 48 hrs at 37^0C. It was observed that biosecurity measures of disinfection, regulated entry, isolated setting of eggs for layers, broilers and duck chick production and separate set of clothing for different units were common practices in those hatcheries. The most cited areas for disinfection were unit floor followed by hatchery walls. Bacterial and fungal load (CFU/90mm diameter petriplate) was significantly ($P<0.05$) higher in hatcher tray and hatcher wall than in other points. Based on colony characteristics, most of the organisms were gram-negative motile rods indicative of *E. coli* and Salmonella with a few gram-positives and *Aspergillus* species. All hatchery units had higher load of bacteria and fungi, indicating less effective biosecurity and hygiene. An improved hygienic practice is recommended in the hatcheries in Chitwan.

Key words: commercial hatchery, poultry, contamination, sample

INTRODUCTION

Commercial hatcheries have been shown to be reservoirs for *E. coli*, Salmonella, Enterococci, Staphylococcus, fungi and many more pathogen. Several studies suggested that a single egg containing a marker strain of Salmonella or *E. coli*, upon hatch, spread the marker pathogen throughout the hatcher (Cason & Bailey, 1994). Hatchery hygiene monitoring is recognized as a most important tool for quality day old chick's production (Chen et al., 2002; Rodgers et al., 2003; Warren et al., 2013). Poor standards of hatchery hygiene may lead poor quality chicks with increased mortality in early life and poor growth resulting in severe losses to a country's poultry industry and in economy (Harry & Gordon 1966; Scott & Swetnam, 1993; Sheldon & Brake, 1991). Production of day old chicks of broilers and layers are increasing day by day but hatchery environment and its operation in Nepal are not well organised and studied well. Poultry production has been steadily expanding in Nepal. By 2016, it is estimated that 2500 thousands day old broilers and 125 thousands day old layer chicks from 94 hatcheries produced every week. Major constraints for quality are lack of adequate training of hatchery operator, poor hygiene and sanitation of hatchery, poor biosecurity, poor monitoring and multi stage incubation. Temperature and humidity of hatchery is very conducive for pathogens, which can adversely affect hatchability of the eggs and can result in embryonic deaths and early

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chick mortality adversely affecting hatchery hygiene and performance (Ayachi et al., 2010; Saif et al., 2003 ; Shane, 1999,). There is no any study and publication related to hatchery hygiene in Nepal till date. Understanding the hygiene status of hatcheries would contribute to a wider knowledge of the magnitude, impact and factors contributing to production of quality day old chicks in Nepal.

For qualitative and quantitative increment of day old chicks require numbers of routine management and interventions. Most critical intervention is monitoring of an effective hatchery sanitation program to know the status and effectiveness of current cleaning and disinfection program and assess the strengths and weaknesses of our particular hygiene and sanitation control programme or system by 'hygiene score' or 'index' for a particular section or hatchery. Further effective measure can be applied at most critical point of production stage.

This study was initiated with the objective of determining the levels of hygiene at commercial poultry hatcheries using microbiological examination of hatchery derived samples as a screening tool to estimate pathogen contamination levels.

MATERIALS AND METHODS

Study area

This study covered seven commercial broilers and three commercial layers hatcheries located in Chitwan, Nepal. All hatcheries were located at distances not >35 km from the centre of the city of study regions.

Data collection

The study was conducted from March to August 2016. The questionnaire incorporated open and closed ended questions designed to gather hatchery level information related to unit operations and hygiene. Questionnaires were extensively piloted and modified to ensure that data quality is maximized. Questions in the survey covered farm/hatchery history, management practices employed with keen attention to disease biosecurity and hygiene. Data collection techniques also included direct questioning and discussions with hatchery operators including a review of hatchery records where possible. Observation was also used to verify data.

Sampling and handling

Hatchery visit was planned to collect sample. Twenty-two samples were collected from each hatchery on Non-selective (nutrient agar), selective and differential media (MacConkey agar) and Sabouraud Dextrose Agar (SDA) plate labeled from different part of hatchery (table 2). To use the exposure plates, simply placed the plate on a horizontal surface, removed the lid for the desired exposure time (e.g. 30mins) and then replaced the lid. Lid of contact (Rhodac) plates was removed and pressed the agar gently against the selected surface to be tested (horizontal or vertical) and the lid was replaced taking care not to touch the agar. All plates were labeled clearly as outlined above. A few drops of prepared antibiotic injectable or vaccine sample were dropped onto an exposure plate and the plate immediately closed and then gently tilted to spread the vaccine over the agar.

Two or more marked "control plates" were subjected to the same conditions, as the test plates but remained unopened and returned to the laboratory. All the collected sample plates were transferred to the laboratory in an icebox.

Reading of contact and exposure plates

Once the plates were received at the laboratory, they were incubated for 24hrs at 37°C, after which they were scored for bacterial and fungal growth according to the table 1. They were then incubated at 30°C for a further 24hrs and then scored for fungal growth. Microbial levels were expressed as colony forming units (CFU per 90mm diameter plate). Arithmetic mean CFU values for specific sites common to all hatcheries were calculated and depicted in tabular form. All plates were scored in the laboratory post incubation for bacterial and fungal growth. The total score was based 50:50 on bacterial and fungal counts. The growth on each plate was ranked and given a score factor. A final hygiene index was then calculated for both bacterial and fungal scores and an overall index was determined.

Table 1. Reading of plates (CFU=colony forming unit)

Bacteria	Score	Fungus/Aspergillus	Grade
No bacterial growth	0	No fungal growth	Excellent
1-10 CFU	1	1-2 CFU	Average
10-50 CFU	2	3-5 CFU	Poor
More than 50 CFU	3	More than 5	Miserable

Data analysis

Data collected were entered and managed in MS Excel. Descriptive statistics was used to calculate the mean CFU of bacteria and fungi. Means were compared by DMRT at 5% level of significance.

RESULTS AND DISCUSSION

Hatchery information

Out of 10 hatcheries (Seven broilers and Three layers hatcheries) studied, 4 hatcheries had more than 40 thousands chicks production per week; 3 had 20-30 thousands (one layers and two broilers hatchery) per week and rest 3 were producing less than 20 thousands per week (Two layers and one broiler hatchery). Most of the hatchery managers were employees, not the owners, and four of them were high-school graduate and three of them just literate. The average age of the interviewed hatchery manager was 49.5 years (range 22-55). Most of the hatcheries (70%; 7/10) started before 2010 and only three started recently (after 2010).

Biosecurity measures

The different biosecurity measures commonly followed by the hatcheries in order to avoid disease occurrence in their units were routine usage of disinfectants (n=8; 80%), strict entry prohibition of non-authorized personnel (n=4; 40%), avoidance of mixing birds of different production purposes together (i.e. layers, broilers, duck n=4; 40%) and change of clothes after each unit operation (n=2; 20%). The most cited targeted areas for disinfection was unit floor (n=10; 100%) and hatchery walls (n=2; 20%).

Microbiology of hatchery sample

Actual mean bacterial and fungal CFU values expressed levels of microbial contamination in various hatcheries. Various published reports have graded to contrast levels of bacterial and fungal load among hatcheries or for spatial and temporal comparison within a facility (Clemmer et al., 1960; Hinshaw et al., 1926). The mean level of aerobic contamination is highest in the hatcher tray, hatcher wall contact sample and significantly higher ($p<0.05$) than other points. The results of air sample and contact sample contamination by aerobic bacteria and fungi are presented in table 2 and 3.

Table 2. Mean bacterial contamination (CFU/90mm diameter petriplate) of contact and exposure plates from different hatchery

Sampling points	Sample/ hatchery	Hatchery										Mean
		A	B	C	D	E	F	G	H	I	J	
Mareks/vaccine as-mixed sample	1	1	0	1	0	0	0	0	1	0	0	0.3^{g}
Egg store air sample	1	7	2	12	15	14	0	8	7	2	6	7.3^{fg}
Setter air samples	6	1.2	3.3	1.2	1.4	1.1	1.1	1.6	2.3	0	1.1	1.4^{g}
Hatcher air samples	4	105.8	81.9	153.1	98.6	133.4	85.7	67.3	118.2	67.4	76.5	98.8^{c}
Hatcher tray contact samples	4	351.5	286.4	303.6	284.4	253.6	288.8	105.3	208.4	205.5	77.8	236.5^{a}
Hatcher wall contact sample	1	341	164	252	303	183	152	152	155	88	65	185.5^{b}
Hatcher ceiling contact sample	1	50	30	40	28	24	32	20	18	23	28	29.3^{defg}
Hatcher fan contact sample	1	54	34	36	54	65	30	39	63	26	37	43.8^{de}
Chick holding room sample	1	55	40	39	55	82	54	60	75	33	53	54.6^{d}
Chick holding room air sample	1	35	43	30	45	60	40	45	44	35	33	41.0^{def}
Delivery truck contact sample	1	18	12	22	27	25	13	8	21	14	9	16.9^{efg}

Note: Mean separated by DMRT and columns represented with different letter (s) are significant

Table 3. Mean fungal contamination (CFU/90mm diameter petriplate) of contact and exposure plates from different hatchery

Sampling points	A	B	C	D	E	F	G	H	I	J	Mean
Mareks/vaccine as-mixed sample	0	0	0	0	0	0	0	0	0	0	0.0^{e}
Egg store air sample	7	2	7	8	8	0	8	3	5	0	4.8^{e}
Setter air samples	2.3	4.4	2.9	1.1	5.7	1.6	2.6	3.5	2.3	1.5	2.8^{e}
Hatcher air samples	5.7	3.7	6.6	3.3	6.9	4.4	3.7	4.8	3.5	5.5	4.8^{e}
Hatcher tray contact samples	58.6	57.2	60.4	35.5	30.1	40.2	50.3	36.2	38.6	46.4	45.4^{b}
Hatcher wall contact sample	56	60	39	49	58	59	63	46	33	46	50.9^{a}
Hatcher ceiling contact sample	2	3	3	4	7	6	5	5	2	3	4.0^{e}
Hatcher fan contact sample	7	5	8	6	2	3	2	3	1	3	4.0^{e}

Chick holding room sample	18	20	16	23	41	26	30	28	32	24	25.8^{c}
Chick holding room air sample	6	4	5	7	2	8	3	5	3	5	4.8^{e}
Delivery truck contact sample	25	11	23	13	9	7	20	6	3	7	12.4^{d}

Note: Mean separated by DMRT and columns represented with different letter (s) are significant at 5% level of significance

Although vaccine should be free from contamination, its presence suggests that there is chance of contamination during chicks processing. Level of fungi in the various sites in all the hatchery were considered to be higher in relation to previous studies conducted with the open plate method and contact plates method. In similar study, contact plate method detects higher degree of microbial contamination than open plate method (Gehan, 2009; Kung's, 2007). Open plates detect only viable microorganism and it may give false impression that the air is clean if most of the air born microorganisms are dead (Kung's, 2007). In contrary to this, Kim & Kim, (2010) found the bacterial contamination on the surface of the equipment and facilities showed similar tendencies with that of air. However, on the surfaces of the equipment and facilities in the hatcher room corridors and non operating hatchers where the bacterial contamination of the air was low, bacterial counts were high, measuring over 100 cfu/16 cm^2. Ernst et al., (1980) reported that the open plate technique appeared to be practical under field conditions. Soucy et al., (1983) found that the open plate air sampling procedure produced ratings, which agreed closely with ratings based on fluff counts.

Kim & Kim, (2010) stated that, in the operating hatchers, the contamination of air by aerobic bacteria, coliform, and fungi was high, measuring over 300 cfu/63.6 cm^2. In the egg sorting room, contamination was moderate, whereas in the remaining sampling sites such as the setter room, candling-transfer room, and chick counting room, contamination was minimal, measuring less than 10 cfu/63.6 cm^2 for aerobic bacteria, 5 cfu/63.6 cm^2 for coliform, and 2 cfu/63.6 cm^2 for fungi. Similar findings were observed in our study too. Most section of hatcher and chicks holding area has significantly higher microbial load leading to the high chance of contamination of day old chick. Hygiene and sanitation of hatcher is critical for hatchery hygiene.

Microbial contamination of the chick processing room in hatcheries was higher than the other sampling sites (Except hatcher), a finding that coincides with those of previous studies (Gehan, 2009; Moubarak, 2007; Rodgers et al., 2003; Sander and Wilson, 1999; Shane, 1993). The greatest level of microbial air contamination in a hatchery occurs at hatching, when dust, fluff and bacteria can become air borne and circulate throughout the hatchery. The observation that air-born bacterial counts were proportional to those of surface swabs suggesting 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 (Davies and Wray, 1994; Magwood, 1964).

Mean score for bacterial and fungal contamination was highest in Hatchery F (2.2) and least found in F, H, J (1.8). Score indicates poor for highest bacterial contamination and average for least contaminated hatchery. The mean score bacterial and fungal was highest in the hatcher tray, hatcher

wall contact sample and significantly higher ($p<0.05$) than other points.

Hatcher section of all hatcheries indicated that miserable condition and need further sanitation and hygiene at this level.

Similarly, fungal score was highest in hatchery E and least in hatchery H (2.2) and J, K (1.8) (table 4). This score indicates poor and average status in highest contaminated hatchery and lowest contaminated hatchery, respectively. Variation in score may be due to the difference in hygiene and sanitation for different hatchery.

Table 4. Total mean score of bacterial and fungal contamination of contact and exposure plates

Sampling points	A	B	C	D	E	F	G	H	I	J	Mean
Mareks/vaccine as-mixed sample	1	0	1	0	0	0	0	1	0	0	0.3[f]
Egg store air sample	1.5	1.5	3.5	3.5	3.5	0	2.5	2	2	1	2.1[d]
Setter air samples	1.5	2	1.5	1.5	2	1.5	1.5	2	0.5	1.5	1.6[e]
Hatcher air samples	4	4	4	4	4	4	4	4	4	4	4.0[b]
Hatching tray contact samples	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5[a]
Hatcher wall contact sample	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5[a]
Hatcher ceiling contact sample	2.5	3	3	3	3.5	3.5	3	3	2.5	3	3.0[c]
Hatcher fan contact sample	4.5	3	3.5	4.5	3.5	3	2.5	4	2.5	3	3.4[c]
Chick holding room sample	4.5	3.5	3.5	4.5	4.5	4.5	4.5	4.5	3.5	4.5	4.2[ab]
Chick holding room air sample	3.5	3	3	3.5	3.5	3.5	3	3	3	3	3.2[c]
Delivery truck contact sample	3.5	3.5	3.5	3.5	3.5	3.5	2.5	3.5	3	2.5	3.3[c]
Average	3.2	3.0	3.2	3.4	3.4	3.0	3.0	3.3	2.7	2.9	

Note: Mean separated by DMRT represented with different (s) are significant at 5% level of significance.

Representing each colony based on morphological type, Gram stain, KOH, Catalase, Oxidation Fermentation test, the bacteria were mostly Gram-negative motile rods indicative of *E. coli* and Salmonella with a few gram-positives mainly Streptococci and *Staphylococcus* spp. According to Chen et al., (2002) most hatcheries in Tiawan had a bacterial count ranging from scale 1 to 3. Among these hatcheries, 13% to 29% were contaminated with *Salmonella spp*.; and 33% to 73% were contaminated with fungi. Bacterial load by hatchery and type of samples are shown in table 5.

Table 5. Enteric pathogens detection rate by sample type (n=59)

Sample	No of samples	Detection rate	
		No. positive	%
Marek's/vaccine as-mixed sample	10	3	30
Egg store air sample	10	9	90
Setter air samples	6X10	48	80
Hatcher air samples	4X10	40	100
Hatching tray contact samples	4X10	40	100
Hatcher wall contact sample	10	10	100
Hatcher ceiling contact sample	10	10	100
Hatcher fan contact sample	10	10	100
Chick holding room sample	10	10	100
Chick holding room air sample	10	10	100
Delivery truck contact sample	10	10	100

Least bacterial growth rate was detected in Marek's/vaccine as-mixed sample (30%), closely followed by setter hall air sample, setter air sample and egg store air sample. 100 percent growth was observed in almost all points of chicks production.

Results of the present study indicate that there was a wide range of bacteria pathogens prevailing in the ten hatcheries surveyed. Identification of *Salmonella* spp, *E. coli* by biochemical test strongly supports the results of other workers (Berrang et al., 1995; Ghosh and Panda, 1998; Mdegella, et al., 2000). The incidence and extent of Salmonella and other enterobacteriaceae group of bacteria revealed by this study was in agreement with findings of Gosh and Panda, (1998). Kim & Kim, (2010) found *Salmonella* was the main isolate from the hatcher rooms, chick counting room, and the related equipment and facilities but not from the areas used for the earlier processing step such as the egg receiving room, egg sorting room, setter rooms, and candling-transfer room. There was greater rate of bacterial recovery from hatcher. Evidence of Salmonella as detected by this study indicates that this pathogen is circulating in some breeding units, which, most logically, would be through transovarian transmission. There are much evidence that some chicks acquire Salmonella and other bacterial pathogens as they hatch from their own contaminated shells and shell membranes (William and Dillard, 1968). The reason for lower incidence of bacterial pathogen positive samples in different hatchery could be due to the size of each respective units and the frequency and number of visitors/operators. For instance, the hatching eggs are gathered frequently because of the size of the flocks and the value of the eggs. Comparatively hatchery H and Hatchery J identified to be the smallest units whereas hatchery A, E and F were considered to be larger, producing more than 40,000 chicks production.

CONCLUSION

The findings of this study showed that hygiene status of hatcheries in Chitwan was poor indicating less effective biosecurity and management. The potential risk is to the health status of day old commercial chicks supplied by the hatcheries. An improved hygienic practice, frequent monitoring and evaluation of hygiene is highly recommended. Further studies should be directed at molecular typing and antimicrobial resistance tests of the isolates.

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REFERENCES

Ayachi, A., Alloui, N., Bennoune, O., & Kassah-laouar, A. (2010). Survey of *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria. *Journal of Infection in Developing Countries,* 4(2),103-106.

Berrang, M.E., Cox, N.A., & Bailey, J.S. (1995). Measuring air borne microbial contamination of broiler hatching cabinate. *Journal of Applied Poultry Research*, 4,83

Cason, J., Cox, N., & Bailey, J. (1994). Transmission of *Salmonella typhimurium* during Hatching of broiler chicks. *Avian Diseases, 38*(3), 583-588. doi:10.2307/1592082.

Chen, S. J., Lee, T. E., Wang, E. M., Cho, T. J., & Wang, C. H. (2002). Monitoring the hygiene of chicken hatcheries in Taiwan during 1999-2001. *Journal of Microbiology, Immunology and Infection,* 35(4), 236-242.

Clemmer, D.I., Hickey, J.L.S., Bridges, J.F., Schliessman, D.J., & Shaffer, M. F., (1960). Bacteriologic studies of experimental air-borne salmonellosis in chicks, *Journal of infectious diseases*, 106, 197-210.

Davies, R.H., & Wray, C. (1994). An approach to reduction of salmonella Infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. *International Journal of Food Microbiology*, 24,147-160.

Ernst, R. A, Click-Smith, J., & Bickford, A. A. (1980). Microbiological monitoring of hatcheries and hatching eggs. Proceedings: Western Poultry Disease Conference and l4th California Health Symposium (29th), 96-100

Gehan, Z. M., (2009). A new approach to evaluate the hygienic condition of commercial hatcheries department of veterinary hygiene and management, *International Journal of Poultry Science,* 8 (11), 1047-1051.

Ghosh, N. N., & Panda, S. N. (1998). Isolation of *Salmonella gallinarum* from Poultry in Orissa. *Indian Veterinary Journal,* 65,1049-1051

Harry, E. G. & Gordon, R. F. (1966). Egg and hatchery hygiene. *Veterinarian,* 4, 5-15.

Haynes, R. L. & Smith, T. W. (2003). Hatchery Management Guide, Retrieved February 25, 2017, from htpp://www.msstate.edu/dept/ poultry/hatchmanagement.htm.

Hinshaw, W.R, Upp, C.W., & Moore, J.M., (1926). Studies in transmission of bacillary white diarrhea in incubators, *Journal of the American Veterinary Medical Association,* 68, 531-641.

Kim, J.H., & Kim, K.S., (2010). Hatchery hygiene evaluation by microbiological examination of hatchery samples, *Poultry Science*, 89 (7), 1389-1398. doi: 10.3382/ps.2010-00661

Kung's, J., (2007). *Taking air samples for mold testing+ settle plate method.* Retrieved September 10, 2016, from http://www.moldbacteriaconsulting.com/fungi/taking-air-samples-for-mold-testing.html

Magwood, S.E. (1964). Studies in hatchery sanitation i.(1) Fluctuations in microbial counts of air in poultry hatcheries. *Poultry Science*, 43, 441-449

Magwood, S.E., & Marr, H. (1964). Studies in hatchery sanitation 2.A simplified method for assessing bacterial population on surfaces within hatcheries. *Poultry science*, 43,1558-1566.

Mdegella, R. H., Mmeta, G. S., Minga, U., & John, E. O. (2000). Molecular epidemiology of *Salmonella gallinarum* in chicken in Tanzania. *Tanzanian Veterinary Bulletin*, 8, 33.

Moubarak, S.T., (2007). Microbiological monitoring of hatchery sanitation. *Egyptian Journal of Biomedical Sciences*, 24, 94-105.

Rodgers, J. D., McCullagh, J.J., McNamee, P. T., Bell, C., Brice N., Smyth, J. A. & Ball, H. J. (2003). An investigation into the efficacy of hatchery disinfectants against strains of *Staphylococcus aureus* associated with the poultry industry. *Veterinary Record,* 153, 656-657.

Saif, Y. M., Barnes, H. J., Glisson, J. R., Fadly, A. M., McDougald, L. R., & Swayne, D. E. (Eds.) (2003). *Diseases of Poultry* (11th ed.). Iowa State University Press, Ames.

Sander, J.E., & Wilson, J.L., (1999). Effective of H_2O_2 disinfection during incubation of chicken egg on microbial levels and productivity. *Avian Disease*, 43, 227-233

Scott, T.A., & Swetnam, C. (1993). Screening sanitizing agents and methods of application for hatching egg I. Enviromental and Friendliness. *Journal of Applied Poultry Research*, 2, 1-6.

Shane S. (1999). Promoting chick quality and liveability, ASA Technical Bulletin PO, 43, 1-3.

Shane, S.M., (1993). Use of reutercentrifugeal air sampler to monitor the microbial level in hatcheries, *Journal of Applied poultry Research*, 2, 249-252

Sheldon, B.W., & J. Brake, (1991). Hydrogen peroxide as an alternation hatching egg disinfectant, *Poultry Science,* 70, 1092-1098.

Soucy, K., Randall, C. J., & Holly, R. A. (1983). Microbiological monitoring of hatchery sanitation. *Poultry Science,* 61, 298-309

Warren, C., Weber, S., & Crespo, R. (2013). Modified technique for hatcher and environmental fluff testing. Proceedings of the sixty-second western poultry disease conference, 30-31.

William, J.E., & Dillard, L. H. (1968). Salmonella penetration of fertile and infertile chicken eggs at progressive stages of inoculation. *Avian Disease*, 12, 629-62