Studies on Pathogens Causing Low Hatchability in Eggs and the Effect of Lactobacillus Acidophilus on Controlling of Salmonella Typhimurium and Proteus.

Fyrouz A. M.[1]; Hassan Eman R.[2] and Rabiee Nagwa S.*[2]

[1] Department of Parasytology and Animal Disease, National Research center, Dokki Giza, Egypt
[2]Department of poultry disease, National Research center, Dokki Giza, Egypt
nagwasrabie@hotmail.com*

Abstract: Trials of isolation of different bacterial strains were done on 1025 samples taken from dead in shell embryos constituting 7 kinds of chicken breeds (Matroh, Dandarawy Fayoumi, Dokki, 4, G. Montaza, Hawarra, and Mandara). Infertile eggs and hatcheries (Dust, waters fluffs sampls also 90 blood samples were collected from 3 kinds of chicken breeds (Fayoumi, Matroh, and Dokki 4), for measuring specific antibody to M. gallisepticum (M.G.) by Enzyme linked immune sorbet assay (ELISA). The most predominant bacteria isolated were Pr.mirabillis and S. typhimurium using of Lactobacillus acidophilus in controlling of Proteus Mirabilis and S. typhimurium infection in one day old chicks was studied, the treatment was under taken at different doses routes of inoculation and course to infected chicks. The criteria used for judgment of therapeutic effect were bacterial reisolation from internal organs of dead chicks and rate of mortalities. Orally administration of lactobacillus acidophilus (0.5ml of $3x10^8$ cfu/ml) with S.typhimurium and lactobacillus acidophilus with Pr.mirabillis (0.5 ml of $1x10^6$ CFU/ml), for two successive days gave the best results with low mortality rates than subcutaneous inoculation.

[Fyrouz A. M.; Hassan Eman R. and Rabiee Nagwa S. **Studies on Pathogens Causing Low Hatchability in Eggs and the Effect of Lactobacillus Acidophilus on Controlling of Salmonella Typhimurium and Proteus.** Report and Opinion 2011;3(2):8-13]. (ISSN: 1553-9873). http://www.sciencepub.net.

Keywords: Pathogens; Hatchability; Egg; Lactobacillus Acidophilus; Salmonella Typhimurium; Proteus; subcutaneous inoculation; Enzyme linked immune sorbet assay (ELISA)

1. Introduction:

Bacterial infection of poultry is representing a world wide important factor in term of their economic losses and public health.

Some organism decrease egg production and lead to high embryonic mortalities, others are widely distributed in hatcheries eggs may be a source of spreading the infection (Safwat et al., 1984 and Choud hury et al., 1993).

Salmonella infections acquired vertically from parents or horizontally in the hatchery caused significant growth depression and mortality in young chicks (Gast and Beard 1990).

Hatcheries play an important role in transmission of salmonella between eggs of different origin especially Salmonella typhimurium, Salmonella infants and Salmonella indiana (Geissler et al. 1982) Proteus mirabilis were isolated from non fertile eggs, dead in shell embryos and dead chicks (Yousseif and Gisler, 1985).

Pseudomonus Aeruginosa (Ps. Aeruginosa) is widely distributed in commercial hatcheries and Balady hatcheries (Karaman, 1980).

Many bacterial agents isolated from dead in shell embryos in E, coli, Salmonella and Proteus Sps., (Al-Sadi et al., 2000) in addition Arizona sp, streptococcus spp., Bacillus Cereus, and Bacillus (Nazer and Safari, 1994).

Poultry bacterial pathogens are mainly controlled by using chemotherapeutics but these pathogens are not easy to be controlled which result infections drug resistance. More ever their use in veterinary medicine represents a potential health hazard for human as they leave a drug residue in poultry products (Hamdy, et al., 1983, Panigraphg et al., 1983 and Kaldhusdal and Hofshagen 1992). So it must be searched about other method of control.

Competitive exclusion and probiotics are naturally control methods that are based on ensuring the bird has an adequate gut microflora to countess the growth and colonization of potentially pathogenic bacteria its digestive tract. They act by lowering the ph. Through production of lactate lactic acid and volatile fatly acids (Mulder, 1996).

Dhingra(1993) reported that the probiotics regulate the microbial environment of the intestine and inhibit the pathogenic micro-organism, Fuller and Turrey (1971) reported that lactobacilli was either bacteriostatic or bactericidal in vitro, Miyamoto et al.,(2000) reported that lactobacillus in the cloacae and vagina hens may have protective effect aganist s. enteritidis colonization.

The present work was designed to isolated the most common bacterial causes of embryonic death in hatcheries and to study bacteriological control of the most communes isolated bacteria S.typhimurim and Pr. mirabillis by using (LBA).

2. Materials and methods

1- Samples for bacteriological examination

For bacteriological examination. A total of 1025 samples including 700 samples from dead in-shell embryos constituting 7 kinds of chicken breeds 175 samples from infertile eggs and 150 samples from hatcheries including 50 samples from dust, 50 samples from watt and 50 fluff samples.

90 serum samples were collected from 3 kindes of chicken breedes for detection of Mycoplasma by ELISA;

2- Isolation and identification of bacterial strains:

a. Samples of dead in shell: Sterile swabs from entire side of egg shell inoculated into MacConkey agar then incubated at 37°C. for 24 hr..
b. Samples of infertile eggs: Aloupful of thoroughly mixed whole content and aloubful of yolk sac were inoculated separately into brain heart infusion broth and tetrathionate broth incubated at 37°C for 24 hr. then streaked on Mac.Conkey agar and Hekton enteric agar media at 37°C for 48 hr.
c. Bacteriological examination of hatcheries water, dust and fluff:

A tea-spoonful from water, dust and fluff were inoculated into brain heart infusion broth .MacConkey broth and selenite broth and incubated at 37ºC for 18hs before streaking onto blood agar, MacConkey agar and Hekton enteric agar media for up to 72hrs at 37ºC.

3. Bacterial strains used in experimental infection:-

1. Salmonella typhimurium :

A concentration of $3x10^{8}$ ^{cFu}/ml of S. typhimurium (OZdemir1996) was used for experimental infection in day old chicks as well as through egg shell in fertile eggs.

2. *Proteus mirabilis* :

A concentration of $1x10^{6}$ cFu/ml of Pr. Mirabilis (Venkanagouda et al., 1996) was used for experimental infection in day old chicks as well as through egg shell in fertile eggs.

3. *Lactobacillus acidophilus* (B.N.L.TB. 002, Micro biotech. MSA):

It was used as competitive exclusion product against challenge with *Salmonella typhimurium* and Pr .mirabilis experimentally inoculated through s/c injection, oral route as well as egg shell penetration.

1-ELISA coating Antigen

It was prepared according to the method supplied by Higgins and Whithear (1986) To the Veterinary serum and Vaccine Institute, Abbasia, Egypt.

It was used for screening of serum samples against M.gallisepticum

Stereotyping of Salmonellae

Cultures that yield reactions similar to those given by *Salmonellae* on TSI; urea media and other biochemical tests should be screened

Serologically; this was accomplished rather easily with the slid plate agglutination test with "O" omnivalent *Salmonella* test sera. If agglutination occurred; they should be tested with polyvalent antisera representing no. (1, 11.111). If agglutination occurred, they should be tested for their individual factor for O and H to determine their groups and species according to Kauffman-White scheme *(Cruickshank et al., 1975)*

Experimental Designs:

Six hundred day-old chicks were divided into 12 equals groups.

Experimental chicks

Six hundred one- day old chicks were used for experimental infection with the most prevalent isolates. These chicks were divided into 12 equal groups

1-Group NO. 1:- chicks were injected s/c with .0.1 ml. of $3x10^{8}$ CFu/ml.of the first microorganism. (*S. typhimurium)*
2-Group NO. 2:-chicks were .inoculated with 0.5 ml of $3x10^{8}$ CFu/ml of saline suspension of the first microorganism under study per os.
3-Group NO. 3:- chicks were injected s/c with 0.1ml of $1x10^{6}$ CFu/ml of second microorganism *P.mirabilis*
4-Group NO. 4: -chicks were inoculated 0.5 ml$1x10^{6}$ CFu/ml of saline suspension of second microorganism under study. Per os.
5-Group NO. 5; and Group NO. 6:-as a control.
7-Group NO. 7:-injected s/c with 0.1ml of $1x10^{6}$ CFu/ml of *Lactobacillus acidophilus* and 0.1 ml of $1x10^{6}$ CFu/ml of the first microorganism under study.
8-Group NO. 8 :-injected s/c with 0.1ml of $3x10^{8}$ CFu/ /ml of *Lactobacillus acidophilus* and 0.1 ml of $1x10^{6}$CFu/ml of the second microorganism..
9-Group NO. 9:-inoculated per os with 0.5 ml of $3x10^{8}$ CFu/ml of *Lactobacillus acidophilus* and 0.1 ml of $1x10^{6}$ CFu/ml of the first microorganism for two successive days.

10-Group NO. 10:- inoculated per os with 0.5 ml of $3x10^8$ CFu/ml of *Lactobacillus acidophilus* and 0.1 ml of $1x10^6$CFu/ml of the second microorganism for two successive days.
11-Group NO. 11:-was injected s/c with 0.5 ml of *Lactobacillus* at a concentration of $3x10^8$ CFu/ml.
12-Group NO. 12:-Was inoculated with 0.5 ml of *Lactobacillus* at a concentration of $3x\ 10^8$ CFu/ml for two successive days per os

Experimental Designs:
Two hundred fifty day-old chicks were divided into 7 equals groups.

Experimental chicks
Two hundred fifty one- day old chicks were used for experimental infection with different typs of vaccines as (H_5N_1, H5N2). These chicks were divided into 7 equal groups
1-Groups were treated with normal program for prevention against other viruses such as (ND, Gomboro, IB,) also treated with different bacterial antibiotics.

3. Results :
Incidence and frequency distribution of micro-organism isolated from dead in shell embryos

Out of 700 samples of dead embryo chick in broilers (616) Were isolate.

The isolated micro organism were pr. mrabillis (10 % (*S. Typhimurium* (10 % (Pr. Vulgariors (7 % (*E-coli, S. galinarum* and Micro cocas each 8%.

Most predominant isolate Pr.mirabillis and S.typhimurium each (50 isolate).

Isolation of M.O. from infertile eggs (table1).

Out of 175 samples, 55 isolated were recorded as Pr.mirabillis and S.typhimurium (3.4% of each) was the most predominat. M. luteus of the (2, 8% each).

Air, water, dust and fluff samples:

Fifty bacteriological samples from each of hatchery dust, water and fluff were examined thoroughly and the following microorganisms were obtained with a total number of 51 bacterial isolates *P. aeuroginosa, M. varians M. urea* and *P. Mirabilis*(4.00% each) were the most predominant isolates followed by *S. mutans, Sar. flava* and *S. epidermidis* (2.0% each). *S avium,* S. *faecalis, Diphtheroid bacilli,* and *Sar. lutea* were isolated with a percentage of 1.3/ each. The lowest microorganisms isolated were *E. coli,* S.*typhimurium, Ps. fluorescens, C. Freundii, Kl. oxytoca, E. aerogenes* and *A. hydrophila* 0.66% each.

Result of ELISA test for detection of antibodies to Mycoplasma galisepticum; in sera,

Out of the total number of examined sera, 40 samples were collected from the Fayoumy flock, 30samples collected from Matroh flock and 20samples from Dokki-4 flock with a total number of 90 serum samples these sera were selected randomly.
The obtained results were as follows:

Thirty-Five (87.5%) of the examined 40 sera collected from Fayoumy flocks were found ELISA test positive ,20 of the examined 30,samples (66.7%) collected from Matroh flock and 12(60.0%) of 20 examined Dokki -4 flock reacted passively with ELISA coating antigen.

Table (1) Results of experimental infection in embryonated chicken eggs

GROUP NO.	M. O.	NO of dipped eggs	Time of dipping	Embryo mortality	
				NO.	%
1	S. typhimurium	50	15 minutes	32	64
2	S. and L. acidophillus	50	15 minutes	23	46
3	L. acid→ 6.hr .Sal	50	15min.→6hr→15 min.	16	32
4	Control	50	15 minutes	4	8
5	Pr. mirabilis	50	15 minutes	29	58
6	Pr. And L. acidophilus.	50	15 minutes	22	44
7	L. acid→6hr.Protus	50	15min.→6hr→15 min.	14	28
8	Control	50	15 minutes	3	6

Results of experimental infection in embryonated chicken eggs

GROUP NO.	M. O.	NO of dipping eggs	TIME of dipping	Embryo mortality	
				NO.	%
1	S.typhimurium	50	15 minutes	32	64
2	S. and L.acidophilus acidophilus	50	15 minutes	23	46
3	L.acid→ 6.hr .Sal	50	15min.→6hr→15 min.	16	32
4	Control	50	15 minutes	4	8
5	Proteus mirabilis	50	15 minutes	29	58
6	Pr. and L.acidophilus.	50	15 minutes	22	44
7	L.acid→6hr.protus	50	15min.→6hr→15 min.	14	28
8	Control	50	15 minutes	3	6

4. Discussion:

Various kinds of microorganisms were isolated from dead-in-shell embryos of examined 7 local breeds Pr.mirabilis and S.typhimurium were isolated in a rate of (10%(for each out of 700 samples (table 1) followed by Pr. vulgaris (9.7%(Ps. Aeruginosa (7.1) E-coli str. gallinarum, M. inteus 8% fro each), prov. Sturti E.colacae (6.9 from each) and Hal.alvei, leminorella grimontii (6.7% for each).

Proteus was isolated from dead-in-shell chicken embryos (Orjaka and Mohan, 1985)

Abd El-Galil and Abd El latif – M.M (1995): Studied bacterial causes of lowering hatchability and early embryonic chicken death in Balady hatcheries. He reported that E. coli was the most common isolate for both fertile eggs (26.4%) and dead in shell embryo (21.9%).

Al-Sadi et al. (2000) isolated E. coli, proteus spp. and Salmonella microorganisms from dead in-shell embryos. –

The microorganisms isolated from infertile eggs out of 175 were Pr.Mirabilis S.typhimurium, Ps.aeruginosa (3.4% for each) followed by Ent. Aergogenes and M. luteus (2.8% for each). Then Pr. Vulgaris, E-Coli, citi frendii and ENT. agglomerans (2.2% for each) then str. Avium and xanthomonas sp. 1.7% these results accords to Al-Aboudi et al. (1992)

Antibodies against M.gallisepticum in sera of three flocks were detected by ELISA (table 3 and 4(the highest number of positive cases were in Fayoumy breed (87.5%) followed by Matroh Breed (66.7 % (and Dokki-4*A60.6 %).

The diagnosis of M. Galliseptics by ELISA were recorded by ELISA were recorded by Eman (2006).

The uses of probiotic (LBA) with s.typhimurium and Pr. Mirabilis in different doses and routes were studied.

The therapeutic effect by using LBA in chickens at doses given orally or subcutaneously (s/c) gps 9 and 10 proved a safe effect on bird as it gave results similar as negative control gps 3.

The mortality rates of in gps 7and 8 which experimental infected chickens with s.typhimurium treated with (LBA) S/C one dose and orally (two successive days) were 44% and 18% respectively the results decrease as compared with salmonella infected by the same routes untreated groups, gps 1&2 (positive control) which their results were 70% and 24% respectively.

While reisolation rates in gp 7 (81.8%) not decreased than gp1 control positive (80) but decreased in gp8 (22.2%) than pg1 (75%).

This means that the effect of LBA is depend on dose and route of administration and decrease effect of S. typhimurim.

Coconnier et al., (2000) reported that L.B.A... Efficiently decreased transcellular pass age of S.typhimurium.

The mortality rates and reisolution rates of Pr. Mirabilis in gps 11&12 were 22%, 16% and 45.5%, 37.5% which decreased as compared with Pr. Mirabilis infected untreated groups gps 4&5 (positive control). Which their results were 24%, 28% and 83.3%, 57.1% these results nearly similar to Radwan and Hassan (2004) who reported that cloacal prolapse was lowered from 60% in Pf. Mirabilis inoculated layers to 40% when Pr. Mirabilis swabbed simultaneously with L. acidophilus and embryonic mortalities were lowered from 68% to be 44% when P. mirabilis and L. acido philus are inoculated concurrently and lowered again t. be 25% when L. acido philus precedes P. mirabilits with 6 houses.

These results means that effect of LA is dose depredate and also depend on route of administration and decreases effect of used bacterial stasis (S. typhimurim and Pr. Mirabilis).

A traditional protiotics such as LA regulates the microbial environment of the intestine and inhibits the pathogenic intestinal microorganisms and improves the feed conversion efficiency (Dhingra, 1993 and Shoeib et al., 1996).

In conclusion, it would appear from these experiments that the use of proprieties, L.B.A. for broiler chickens could significantly reduce S.typhimurium and Pr. Mirabilis infection and can greatly assist in the control of this infection in native breed chickens.

Corresponding author
Rabiee Nagwa S.
Department of poultry disease,
National Research center,
Dokki Giza, Egypt
nagwasrabie@hotmail.com*

5. References:

1. Abd. El. Galil El- Kenawy- and Abd El latif – M.M (1995): Bacterial cases of lowering hatch ability and early embryonic death in Balady hatcheries- Assiut- veterinary Research Medically journal 33.66, 199.206.
2. Al-Sadi, H.I.; Basher, H.A. and Ismail, H.K. (2000): Bacteriologic and pathogenic studies on dead in shell chicken embryos. Journal article. 1607-3894.
3. Bassuoni, A, A. Saad F.E. Awaad, M.H, H; Shalaby.N.A. and Choudhury- B, chanda- A; Dodgupta- P, Dutta RK and Lila saha(1993): Studies on yolk sac infection in poultry Indian- journal of Animal health 32: 1, 21-23.
4. *Cruick Shank, R., Dugiud, J. P; Marmion, B. P and Swain R. H. A (1975):* Text book of medical microbiology 11th EDT. The English language book society and shurchill living stone Edinburgh and New York.
5. Coconnier; MH, Lievin, V, Lorrot, M, Servin, AL (2000); Antagonistic activity of lactobacillus against intracellular Salmonilla enterica, serovar, Tybhimurium infecting human enterocyte-like Applied and environmental microbiology,66;3;1152,1157.
6. Dhingra, M.M. (1993) Probiotic in poultry diet poultry advise.26 (8); 45.
7. Eman, R. (2006); Studies on pathogens causing low hatchability in eggs; ph D. Fac. Vet. Med., Cairo Univ.
8. Fuller, R.and Turvey, A. (1971); Bacterial associated with the intestinal wall of the Fowls. J.Appl.bact, 34,617-622.
9. Gast, R. K. and Beard, C. W. (1990): Serological detection of experimental *Salmonella enteritidis* infections in laying hens. Avian Dis., (34), 721-728.
10. Geissler- H; Ilescas- S and Kosters- J (1982): Role of deliveries and transmission of *Salmonella* in one day old broiler chicks. Berliner and Munchener, 95: 21, 424- 426.
11. Hamdy, A.H. Thomas, R.W.; Yancey, R.j.and Davis; R.B. (1983); Therapeutic effect of Lincomycin concentrations in drinking water on necrotic enteritic in broilers.poultry, sci.62; 589-591
12. Kaldhusdal; M. and Hofshagen; M ;(1992); Barley inclusion and avoparcin supplementation in broiler diets –clinical pathology and Bacteriological finding in a mild form of necrotic entiritis.poult.sci.71; 1145-11
13. *Karaman, Rokia A. A. (1980):* Studies on some bacterial diseases of poultry causing high mortality in balady hatcheries in Monofia Province. M. V. sc. Thesis (poultry Dis.) Fac. Vet. Med. Cairo Univ.
14. Miyamoto, T.Horrie, T; Fukata, T; Sasi.K. (2000); Lactobacillus flora in the cloacae and vagina of hens and its inhibitory Activity against salmonella enteritidis in vitro poult.sci.79 (1):7-9.
15. Mulder, R.W.A.W. (1996); Probiotics competitive exclusion microflora against Salmonella. World poultry; misset Moy, 30-32
16. *Nazer. And Safari- GH (1994):* Bacterial flora from dead- in shell chicken embryos and their drug resistance. Indian- Journal of Animal Science 64: 10, 1006- 1009.
17. Orjaka,L.S.E. and Moham (1985): Aerobic bacterial flora from dead in shell chicken embryos. Journal article, Avian disease, 26 (3-5):86.
18. Panigraphgy; B; Gyimph; J.E.Hall, C.F.and Williams, J.D. (1983): Immunogenic potency of an oil –emulsified Escherichia coli bacterium. Avian Dis.28; 475-481.
19. Radwan. I. A. and Hassan W.H. (2004): Competitive exclusion of Proteus causing cloacal prolapse and embryonic chicken deaths. Peni-suif Vet. Med, 14 (1); 29-37.
20. Safwat- EEA, El Bakry- M; Mostafa. F and El Sawah (1984): Studies on the antibiotic resistance of *Salmonellae* isolate from poultry and their virulence to chickens. Egyptian- Journal of Animal production publs 24, 1: 2, 295: 301

21. Youssef,Y.I. and Gissler (1985): Experimental infection of chicks with Salmonella Arizona. Avian pathology, 8(2): 163-17.

12/1/2010