Study on the effect of some bacterial strains on IB viral infection

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Abstract: The present study was designed to throw the light on isolation of some bacterial stains from poultry farms like *Salmonella* sp. and *E. coli,* classification and serotyping of bacterial isolates were done, isolation and identification of respiratory viruses (IBV) from poultry farms and studying the effect of isolated bacterial strains on selected respiratory virus. For that purpose, birds showing symptoms of respiratory diseases and general illness were collected for investigating co-infection of different respiratory viral infection with *E. coli* and *Salmonella* infection in different flocks (broilers and layers). The birds were obtained from both private and governmental farms during the period from 2016 – 2018 where a total of 200 broiler and layer flocks (200 sample) were examined (organs and swabs were collected aseptically to prevent cross contamination). The collected organs were cultured within a time limit which did not exceed 24 hours from collection. Results revealed that the prevalence of *E. coli was 67.5%* and *Salmonell*a was 11%, while IBV was 61.5% in the collected samples. The serological identification showed that the most predominant serotype for *E.coli* was O 158 and for *Salmonell*a was S. kentuckey. Also the experimental results revealed that the co-infected groups (IBV+ bacteria) have a higher viral shedding than group infected with virus alone.

[Ashraf A. Abd El Twab, Saad S. A. Sharawi, Soad A. Nasef, Fatma I. El-Hofy, Ahmed Sedeek. **Study on the effect of some bacterial strains on IB viral infection.** *Nat Sci* 2018;16(6):80-90]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 12. doi:10.7537/marsnsj160618.12.

Keywords: EMB, *E. coli*, *Salmonell*a, IB, coinfection

1. Introduction

Diseases of the respiratory tract are a significant component of the overall disease incidence in poultry. In many cases, respiratory disease observed in a flock may be a component of a multisystemic disease or it may be the predominant disease with lesser involvement of other organ systems. In some cases, such as infectious coryza or infectious laryngotracheitis, the disease may be limited to the respiratory system, at least initially. Various pathogens may initiate respiratory disease in poultry, and including a variety of viruses, bacteria, fungi and Environmental factors may augment these pathogens to produce the clinically observed signs and lesions **(Glisson**, **1998).**

Several avian viruses have a predilection for the respiratory tract of chickens: infectious bronchitis virus (IBV), Newcastle disease virus (NDV), infectious Laryngotracheitis virus (ILTV), Avian influenza virus (AIV), and Pneumovirus primarily infect the respiratory tract of chickens. Other viruses, such as Adenovirus and Reovirus, are generally considered to be secondary invaders of the upper respiratory tract of chickens. Pneumovirus and ILTV have been found in tissues of the respiratory tract, whereas IBV, NDV, and AIV also invade other tissues such as the kidneys and the reproductive system (IBV), the gastrointestinal tract (NDV, IBV, and AIV), and the central nervous system (NDV, AIV) **(Villegas, 1998)**.

Infectious bronchitis virus (IBV) which is single stranded positive sense, enveloped RNA virus **(Lai and Cavanagh, 1997)**. The virus has been classified under the *Gammacoronavirus* genus in the family *Coronaviridae*, Order *Nidovirales*. Like other members of *coronavirus* family, the IBV genome is composed of structural and nonstructural proteins. Infectious bronchitis virus (IBV) is one of the major economically important poultry viral diseases distributed worldwide.

It affects both galliform and nongalliform birds. Its economic impact includes decreased egg production and poor egg quality in layers, stunted growth, poor carcass weight, and mortality in broiler chickens. Although primarily affecting the respiratory tract, IBV demonstrates a wide range of tissues tropism, including the renal and reproductive systems. Thus, disease outcome may be influenced by the organ or tissue involved as well as pathotypes or strain of the infecting virus **(Bande *et al.*,2016)**.

E. coli strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC). Colibacillosis is a disease of severe economic significance to all poultry producers

worldwide and is characterized by a diverse array of lesions **(Dziva and Stevens, 2008).** These lesions varied betweenperihepatitis, airsacculitis and pericarditis, or other syndromes such as egg peritonitis, salpingitis, coligranuloma, omphlitis, cellulitis and osteomyelitis/ arthritis **(Barnes and Gross, 1997).**

On the other hand, *Salmonella* infection in poultry caused by a variety of *Salmonella* species and considered as one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced production. Avian *Salmonella* infection may occur in poultry either acute or chronic form by one or more member of genus *Salmonella*, under the family *Enterobacteriaceae*. Besides, motile *Salmonella* (paratyphoid group) infection cause salmonellosis in chickens and have zoonotic significance **(Hofstad *et al.*,1992).**

The interaction between Bacteria and virus was found and reported in several studies (**Mancini *et al.*, 2005**).

E. coli is one of the factors that may affect the pathogenicity of avian H9N2 influenza virus. This bacterium is categorized based on its somatic (O) and flagellar (H) antigens. Any local or generalized infection caused by avian pathogenic *E. coli* O2 is called colibacillosis, the most common bacterial infection in poultry **(Saif *et al.*, 2008).** Colibacillosis is usually seen with other infections resulting in disease deterioration. Simultaneous infection of colibacillosis with paratyphoid and histomoniasis results in high mortality. Colibacillosis with Gamboro cause increase in mortality **(Okiki and Oghbimi, 2008).**

Co-infection of *E. coli* with infectious bronchitis virus (IBV) may lead to a more complex outcome, usually associated with high morbidity and mortality. Similarly, infection with nephropathogenic IBV strains may result in pale, swollen, and mottled kidneys **(Boroomand *et al.*, 2012).**

Another study was conducted to construct and evaluate several recombinant live attenuated *Salmonella* vaccine for their ability to stimulate systemic and humoral responses and protect against direct Avian Influenza virus challenge (**Layton *et al.*, 2009**).

Also it was found that Bacteria present in the respiratory tract are potential sources of proteases that could contribute to cleavage influenza virus in vivo. Susceptibility of HA to cleavage by host intracellular proteases is the major factor distinguishing highly virulent avian influenza viruses from a virulent avian and mammalian viruses **(Mancini *et al.*, 2005)**.

Therefore, the present study was planned out to throw a light on the interaction and the effect of some isolated bacterial strains (*Salmonella* and *E. coli*) on IBV.

2. Materials and Methods.

Sample collection.

Birds showing symptoms of respiratory diseases and general illness were collected and submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production, Dokki, for investigating co-infection of different respiratory viral infection with *E.coli*and *Salm-onella*infection in different flocks (broilers and layers). The birds were obtained from both private and governmental farms during the period from 2016 – 2018. Where a total of 200 broiler and layer flocks (200 samples) examined Organs and swabs were collected aseptically to prevent cross contamination. The collected organs were cultured within a time limit which did not exceed 24 hours from collection.

Bacterial isolation and identification.

E.coli.

The internal organs included liver, spleen, heart and other organs were collected weighed, pre-enriched with buffered peptone water as a 1:10 dilution and incubated at 37^{o}C± 1^{o}C for 18 h. For the isolation of *E. coli,*10 µl loop-full from the inoculated and incubated pre enrichment culture were streaked on the surface of Tryptone soya Agar (TSA), MacConkey agar, Eosin methylene blue agar (EMB) and Xylose Lysine Deoxycholate agar (XLD agar) and incubated at 37.0 ± 1^{o} C for 24 hrs.

Typical *E. coli* colonies grown on XLD agar medium showed yellow colonies, pink colonies on MacConkey agar, and on EMB agar colonies showed dark green metallic sheen. The suspected colonies were examined for their colonial morphology, picked up and examined microscopically by Gram's stain. Pure cultures of the isolates were subjected to biochemical test to identify and differentiate between members of *Enterobacteriaecae*according to **(Quinn *et al.*,2002).**

Serological identification of *E. coli* isolates.

Typing of *E. coli* isolates was performed by the slide agglutination test using standard polyvalent and monovalent *E. coli* antisera. Only fresh bacterial cultures from 24 hours colonies onto nutrient agar media were used **(Edwards and Ewing, 1972)**.

Salmonella.

Samples of collected organs were suspended in Buffered Peptone water as 1:10 dilution and then incubated at 37ºC ±1ºC for 18hrs ±2hrs.

From the pre-enrichment culture, 0.1 ml was transferred to a tube containing 10 ml of the Rappaport- Vassiliadis broth and then incubated at 41.5ºC ± 1ºC for 24hrs ± 2hrs. One ml of the pre-enrichment culture was also transferred to a tube containing 10 ml of the Muller–Kauffmann

tetrathionatenovobiocin broth, and then incubated at 37ºC for 24hrs ± 2hrs.

From the enrichment culture,10 µl were inoculated onto the surface of Xylose Lysine Deoxycholate (XLD), MacConkey and H E agar, then incubated at 37ºC ±1ºC for 24hrs ± 2hrs. The suspected colonies were examined for their colonial morphology, picked up and examined microscopically by Gram's stain. Pure cultures of the isolates were subjected to biochemical test to identification and confirmation (**Murray *et al.*, 2003).**

Serological identification of *Salmonella* isolates.

Diagnostic poly and monovalent "O" and "H" antisera were used for serological identification of *Salmonella* isolates. Only fresh bacterial cultures from 24 hours colonies onto nutrient agar media were used. Typing of *Salmonella* isolates was performed in the Central Laboratory for Veterinary Quality Control on Poultry Production by **Kauffmann and Das Kauffmann (2001).**

Determination of antimicrobial susceptibility profiles for *Salmonella* and *E. coli* isolates (CLSI, 2007).

At least three to five well isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture was incubated at 35°C until it achieved or exceeded the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours). The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml to perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland' standard against a card with a white background and contrasting black lines. Then a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. The antibiotic discs were applied into the plates using sterile forceps then pressed gently onto the surface of the agar using the forceps. Discs were arranged at least 15mm from the edge of the plate and 15-20 mm apart from each others.

The plates was inverted and placed in an incubator set to 37 °C for 16 -18 hours. The diameters of inhibition zones were measured then recorded and interpreted according to **(CLSI, 2007).**

Viral isolation.

The collected organs (Trachea, lung and kidney) were washed in sterile saline, and then frozen at below-10°C. After thawing, the tissue homogenates (10% w/v) were suspended in sterile saline (0.85%w/v) containing 1000 IU/mL penicillin, 1.0 mg/ml streptomycin. By disrupting organs using sterile mortar and pestle, the homogenates were then centrifuged at 3000 rpm for 10 min, and the supernatant was further passed through 22 µm membrane filter for clarification. Also the trachea of live birds was swabbed by inserting cotton swab into the trachea and gently swabbing the wall, and the swab was placed in transport media. The trachea of dead birds was swabbed after the lungs and trachea has been removed from the bird. The trachea was held in a gloved hand and the swab inserted to its maximal length with vigorous swabbing of the wall. This material was examined for presence of virus by real time- PCR. For virus propagation the positive samples were inoculated in 9-11-days-old SPF embryonated chicken eggs via the allantoic sac route and incubated till 6 days post inoculation, then the allantoic fluid was harvested. The harvested fluids were diluted 1:10 in sterile saline containing antibiotic and re-passaged for additional two passages (Second and third) (Some of IBV field isolates were not embryo- adapted and did not cause death or produce lesions on the first passage (**Gelb and Jackwood, 1998**), then collected fluids containing virus were ten fold diluted and inoculated in SPF embryonated chicken eggs for virus titeration according to **(OIE, 2008).** Image (1).

Experimental materials and Design:

Experimental Chickens:

One hundered of one-day-old chicks were hatched from SPF fertile chicken eggs obtained from Nile SPF (KoomOshiem, Fayoum, Egypt), incubated and hatched. Chicks were reared in HEPA-filtered negative pressure isolation units, previously cleaned and disinfected. Chicks were provided with commercial broiler ration, water and feed were provided *adlibidum* till 21 day old.

Viral inoculum strain:

Challenge IB virus:

The virus used in the challenge was in form of infectious allantoic fluid at the level of fifth–passage; they were isolated in RLQP from field cases confirmed by RRT-PCR used in experiment. It was titrated in SPF EGE as described by **Villegas and Purchase, (1990)**, with titer (10^6EID_{50}) and its calculation according to the method of **Reed and Muench, (1938)**.

Bacterial inoculum strains:

***E.coli* inoculum:**

E. coli strain O158 was isolated from field cases and prepared to inoculate by (10^8cfu/ml per bird) IT route.

***Salmonella* inoculum:**

S. Kentuckywas isolated from field cases and prepared to inoculate by (10^8cfu/ml per bird) IT route.

Fifty chickens at age of 21th day were divided into five groups of 10 birds in each.

Group 1: birds were inoculated via intratracheal route with selected virus strain (IBV 10^6 EID50/bird) alone.

Group 2: birds were inoculated via intratracheal route with selected virus strain (IBV 10^6 EID50/bird) and then after 4 days *E. coli* (10^9 colony-forming units).

Group 3: birds were inoculated via intratracheal route with selected virus strain (IBV10^6 EID50/bird) and then after 4 days *Salmonella* sp (10^9 colony-forming units).

Group 4: birds were inoculated via intratracheal route with selected virus strain (IBV 10^6 EID50/bird), and then after 4 days *E. coli* and *Salmonella* sp (10^9 colony-forming units).

Group 5: negative control group

Finally a pooled sample of tracheal swabs from each bird in all groups were taken at 6, 8 and 10 day post inoculation for checking the presence of viral shedding by RT-PCR.

3. Results

Result of bacterial isolation and serological identification.

E.coli.

The prevalence of isolation of *E.coli*reached 67.5% from the examined 200 samples showing symptoms of coli septicaemia. The incidence of *E. coli* infection was 120 samples out of 160 samples with a percentage 75% in broiler and 15 out of 40 samples with a percentage 37.5% in layers. Table (1).

Table (1) Prevalence of *E. coli* recovered from broilers and layers flocks.

Type of flocks	Number of examined samples	Number of +ve samples	% of +ve samples
Broilers	160	120	75
Layers	40	15	37.5
Total	200	135	67.5

Prevalence of *E. coli* strains in isolates from broilers and layer flocks.

Table (2): Prevalence of *E. coli* strains in isolates from broilers and layer flocks

Serotype	Serotype number	Percentage of serotype
O158:k-	18	13.3
O25:k11	15	11.1
O26:k60	11	8.1
O78:k80	10	7.4
O44:k74	9	6.7
O114:K90	9	6.7
O119:k69	8	6
O103:K-	8	6
O127:K63	7	5.2
O125:K70	7	5.2
O86:K-	6	4.4
O91:K-	5	3.7
O111:K58	4	3
O8:K50	3	2.2
Untypaple	15	11.1

Serotyping of 135 *E.coli* isolates was applied by slide agglutination test using polyvalent and monovalent O *E.coli* antisera. 14 different serotypes were identified among *E.coli* isolates and the most predominant one was serotype O158:k- with 13.3% followed by O25:k11,O26:k60 and O78:k80 with 11.1%, 8.1 and 7.4 % respectively. O44:k74 and O114:k90 have the same percentage which was recorded as 6.7%, also O119:k69 and O103:K- have the same percentage which was 6%.It was found that o127:k63 was similar to O125:K70 where both of them were 5.2%,while O86:K-, O91:K- and O111:K58 were recorded as 4.4%, 3.7% and 3% respectively. In contrast O8:K50 had the lowest percentage which was recorded as 2.2%. On the other hand 11.1% of isolates were untypable this showed in table (2).

Salmonella.

Prevalence of *Salmonella* recovered from different flocks.

Table (3) Prevalence of *Salmonella* recovered from broilers and layers flocks.

Type of flocks	Number of examined samples	Number of +ve samples	% of +ve samples
Broilers	160	7	4.4
Layers	40	15	37.5
Total	200	22	11

The prevalence of isolation of *Salmonella* reached 11% from the examined 200 samples showing symptoms of Salmonellosis. The incidence of *Salmonella* infection was 7 samples out of 160 samples with a percentage 4.4% in broiler and 15 out of 40 samples with a percentage 37.5% in layers. Table (3).

Prevalence of *Salmonella* strains in isolates from broilers and layer flocks.

Serotyping of 22 *Salmonella* isolates was appliedand resulted in 4 different serotypes. Among *Salmonella* isolates *S. Kentucky* was the most predominant with 40.9%, *S. Enteritidis* and *S. Infantis*were 27.3% and 18.2% respectively. In contrast *S. Dublin* had the lowest percentage with13.6%. Table (4).

Table (4): Prevalence of *Salmonella* strains in isolates from broilers and layer flocks.

Serotype	Serotype number	Percentage of serotype
S. Kentucky	9	40.9
S. Enteritidis	6	27.3
S. Infantis	4	18.2
S. Dublin	3	13.6

Results of antimicrobial susceptibility

E.coli.

Susceptability of *E.coli*isolated from broilers and layers chicken showed that (86%) of isolates were susceptible to Ciprofloxacin followed by (54%) to Norfloxacin, then (41.6%) to Levofloxacin which considered as most effective antimicrobials. also Susceptability to Sulphamethox-azole/trimethoprim, Chloramphenicol and Gentamycin were (35.8%), (28.3%) and (23.3%) respectively. Either Susceptability was (15.8%) to Ampicillin and (11.6%) to Nitrofurantin. While it was (10.8%) for Tetracycline and (8.3%) for Doxycycline. table (5).

Table (5) Sensitivity percentage of *E.coli* serotypes to each antimicrobial

Antimicrobial agents	Serotype percentage
Ciprofloxacin	**103**/120(86%)
Norfloxacin	**65/120(54%)**
Levofloxacin	**50/120(41.6%)**
Sulphamethoxazole/trimethoprim	**43/120(35.8%)**
Chloramphenicol	**34/120(28.3%)**
Gentamycin	**28/120(23.3%)**
Ampicillin	**19/120(15.8%)**
Nitrofurantin	**14/120(11.6%)**
Tetracycline	**13/120(10.8%)**
Doxycycline	**10/120(8.3%)**

Meanwhile, a high level of resistance of *E.coli*isolates was recorded to doxycycline (67.5%) and tetracycline (65%) followed by Nitrofurantin (64.2%), ampicillin (58.3%), Chloramphenicol (41.7%) and Gentamycin (39.2%). Also resistance to Levofloxacin, Sulphamethoxazole/trim-ethoprim, Norfloxacin and Ciprofloxacin was recorded as (37.5%), (35%), (25%) and (6.7%) respectively as shown in table (6).

Table (6) Resistance profile of each antimicrobial against *E.coli* isolates from chicken:

Antimicrobial agents	Serotypepercentage
Doxycycline	**81**/120(67.5%)
Tetracycline	**78/120(65%)**
Nitrofurantin	**77/120(64.2%)**
Ampicillin	**70/120(58.3%)**
Chloramphenicol	**50/120(41.7%)**
Gentamycin	**47/120(39.2%)**
Levofloxacin	**45/120(37.5%)**
Sulphamethoxazole/trimethoprim	**42/120(35%)**
Norfloxacin	**30/120(25%)**
Ciprofloxacin	**8/120(6.7%)**

Salmonella.

Susceptibility of *Salmonella* isolated from broilers and layers chicken showed that (86.4%) of isolates were susceptible to Ciprofloxacin followed by Levofloxacin (72.2%), and Gentamycin (68.2%) then Norfloxacin (54.5%) which considered as the most effective antimicrobials. Also Susceptibility to Nitrofurantinand Streptomycin were the same (50%), Sulphamethoxazole/trimethoprim and Chloramphenicol were (41%) and (36.4%) respectively. Susceptibility to Tetracyclinewas (18.2%). Also it was the same to Ampicillin and Doxycycline where it was recorded as (13.6%) table (7).

Table (7) Sensitivity percentage of *Salmonella* serotypes to each antimicrobial

Antimicrobial agents	Serotype percentage
Ciprofloxacin	19/22(86.4%)
Levofloxacin	**16/22(72.7%)**
Gentamycin	**15/22(68.2%)**
Norfloxacin	**12/22(54.5%)**
Streptomycin	**11/22(50%)**
Nitrofurantin	**11/22(50%)**
Sulphamethoxazole/trimethoprim	**9/22(41%)**
Chloramphenicol	**8/22(36.4%)**
Tetracycline	**4/22(18.2%)**
Doxycycline	**3/22(13.6%)**
Ampicillin	**3/22(13.6%)**

Meanwhile, a high level of resistance of *Salmonella* isolates was recorded to Tetracycline (63.6%) and Doxycycline (65%) followed by ampicillin (54.5%). Resistance to Sulphamethoxazole/trimethoprim was recorded as (45.5%). While it was the same for Nitrofurantin and Chloramphenicol (27.3%). Resistance to Norfloxacin

was (18.2%) which was the same for Streptomycin. Whereas it was (13.6%) for both of Gentamycin and Levofloxacin. On the other the lowest level of resistance was recorded to Ciprofloxacin where it was (9%) as shown in table (8).

Table (8) Resistance profile of each antimicrobial against *Salmonella* isolates from chicken:

Antimicrobial agents	Serotype percentage
Tetracycline	14/22(63.6%)
Doxycycline	**12/22(54.5%)**
Ampicillin	**11/22(50%)**
Sulphamethoxazole/trimethoprim	**10/22(45.5%)**
Nitrofurantin	**6/22(27.3%)**
Chloramphenicol	**6/22(27.3%)**
Norfloxacin	**4/22(18.2%)**
Streptomycin	**4/22(18.2%)**
Levofloxacin	**3/22(13.6%)**
Gentamycin	**3/22(13.6%)**
Ciprofloxacin	**2/22(9%)**

Result of viral isolation.

Prevalence of IB virus recovered from different flocks.

The result of virus isolation by real time PCR revealed that 103 out of 160 examined samples were positive for IB virus with a percentage of 64.3% in broilers, while in layers 20 samples out of 40 were positive for IB virus with a percentage of 50%. Meanwhile the total percentage of isolation was 61.5%Table (9).

Table (9) Prevalence of IB virus recovered from different flocks.

Type of flocks	Number of examined samples	Number of +ve samples	% of +ve samples
Broilers	160	103	64.3
Layers	40	20	50
Total	200	123	61.5

Result of viral shedding in experimental infection.

Results revealed that mixed infection associated with a higher incidence of IB shedding comparing to single IB infection. However within mixed infection there is marked differences in viral shedding, where group infected with IB then *E.coli*a higher early and prolonged shedding comparable to other infected groups Table (10).

Table (10) mean of virus shedding at 6th, 8th and 10th post viral inoculation.

Group	6 day	8 day	10 day
IB	7.986667	0.419933	0.085547
IB+SAL+E.coli	15.27667	8.589667	0.425667
IB+E.coli	15.92833	10.73033	7.480333
IB+SAL	14.698	6.016333	0.2558

IB= infectious bronchitis virus SAL= *Salmonella*

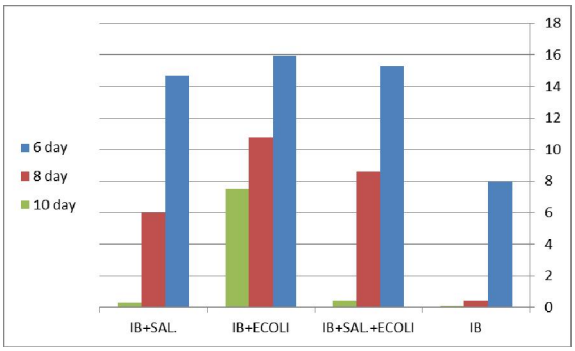

Fig (1): virus shedding at 6th, 8th and 10th post viral inoculation.

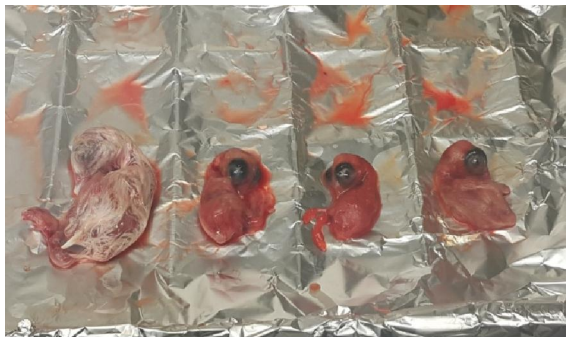

Fig (2): Dwarfing and curling of inoculated embryos with IBV on right side compared with negative control one (non inoculated with IBV) on left side.

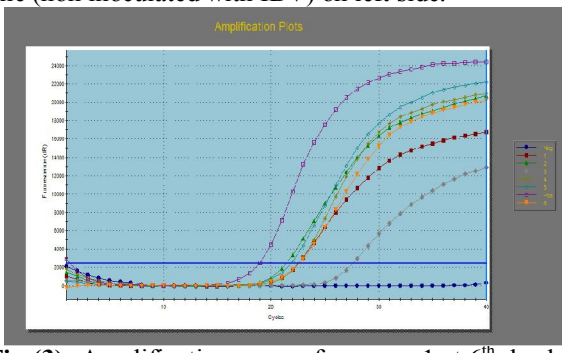

Fig (3): Amplification curves for group 1 at 6th day by real time PCR.

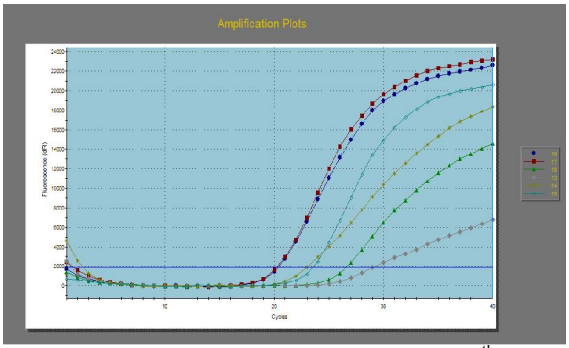

Fig (4): Amplification curves for group 2 at 6th day by real time PCR.

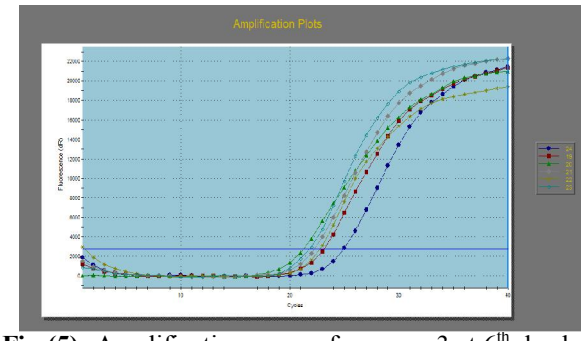

Fig (5): Amplification curves for group 3 at 6th day by real time PCR.

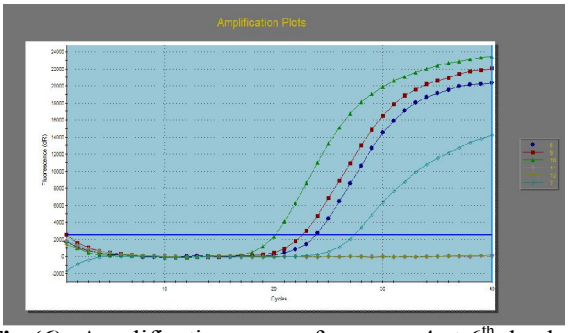

Fig (6): Amplification curves for group 4 at 6th day by real time PCR.

4. Discussion

The present study aimed to study the effect of *E.coli* and *Salmonella* on IBV shedding.

In the present work it was recorded that the prevalence of isolation of *E.coli*reached 67.5% from the examined 200 samples showing symptoms of coli-septicaemia. Almost similar percentages 67% in chickens were reported by (**Syuhada *et al.*, 2013)**, and **(Stella *et al.*, 2016)** reported that From the 80 sampled birds, 48 (60%) *E. coli* was detected of them. Whilea higher incidence incidence of 85.2% was reported by **(Wani *et al*., 2004),** and (**Albarri *et al.*, 2017**) where it was (93.75%).

On the other side a lower incidence (20.5%) was recorded by **(Saidi *et al.*, 2013)** and (43.1%) by **(Roshdy *et al.*, 2012)**.

The result of serotyping revealed that, the most commonly isolated serotypes were O158, O26,O78,O44,O114,O119,O103,O127,O125,O86, O91and O111. These result mostly similar to another study was conducted by **(Roshdy *et al.*, 2012).** Also **(Abd El Tawab *et al.*, 2015)** reported that the most commonly serogroups isolated from chickens were O44, O158, O125 and O103.

In the present study the susceptibility of *E.coli*isolated from broilers and layers chicken showed a high sensitivity to Ciprofloxacin. This finding is similar to previous studies conducted by (**Guerra *et al.*, 2003**) and (**Hasan *et al.*, 2011**) which found that *E. coli* isolates were highly sensitive to ciprofloxacin. The high sensitivity to ciprofloxacin might be because it is a broad spectrum antibiotic that is still relatively new and has limited use by poultry farmers. Unlike **(Omer *et al.*, 2010)**, who found that avian *E. coli* isolates were highly resistant to ciprofloxacin. The data of this study showed that there was a high level of resistance against doxycycline, tetracycline and ampicillin. This is go a hand with a several reports **(Sharada *et al.*, 2009)** and (**Zakeri and Kashefi, 2012).** The presence of high resistance is probably due to the increased use of antibiotics as feed additives, for example, tetracyclines, bacitracin, and cloxacillin are widely used in poultry industries for growth promotion or prevention of diseases **(Omer *et al.*, 2010)**.

It was recorded that the isolation of *Salmonella* reached 11% from the examined 200 samples collected from broilers and layers showing signs of illness. Almost similar results were recorded by **(Ozbey and Ertas, 2006)** and **(Osman *et al.*, 2014)**. Whilst a higher prevalence was recorded in several previous studies by **(Bai *et al.*, 2015)** 45.2% and likewise **Asif *et al.*, (2017)** which reported as 23.3%. On the other hand lower prevalence of *Salmonella* isolates was recorded by **Mir *et al.* (2015)** where it was 6.31%. And **Shekhar *et al.*, (2013)** which was 0.94%.

The present data recorded the prevalence of *Salmonella* in chicken was 11%. They were serotyped into *S.* Kentucky (40.9%), *S.*Enteritidis (27.3%), *S.* Infantis (18.2%) and *S.* Dublinhad the lowest percentage with (13.6%), indicating predominance of *S.* Kentucky. This roughly similar to several studies conducted by **Santos *et al.* (2007)** and **Alambedji *et al.* (2006)** who indicated that the most prevalent serotype were serovar *S.* Kentucky. While there were different results mentioned by **Zhao *et al.*, (2016)** and **Bai *et al.* (2015)** who found that the most common serotype isolated from chicken was *S.* Enteritidis.

Concerning, the result of susceptibility of different *Salmonella* serotypes in the recent work it was found that, there was a high sensitivity to ciprofloxacin, levofloxacin and gentamycin. This mainly agreed with previous results recorded by **Naik *et al.* (2015)** and **Fardsanei *et al.* (2016).** However **Hassan *et al.* (2016)** recorded a high resistance to ciprofloxacin.

The present work revealed that there was a high resistance of different *Salmonella* serotypes to tetracycline. This echoes previous results by **Li *et al.* (2017)** and **Hur *et al.* (2011)** where a high resistance to tetracycline was recorded. In contrast to the present study **Thung *et al.* (2016)** recorded that all *Salmonella* isolates were sensitive to tetracycline.

Concerning to viral isolation. A total 123 samples out of 200 were positive for IBV isolation

(61.5% of the samples), where 64.3% and 50% of the samples were positive for broilers and layers respectively. Almost similar results were recorded by **Roussan *et al.* (2008)** and **Zanaty. (2014).**

While a higher percentage reached to 88% recorded by **Abdel-ELGhany *et al.* (2015).** On the other hand a lower isolation percentage was recorded by **Mohamed and Ibrahim. (2015)** which was 14.28%.

In the present work the effect of some bacterial strains (*Escherichia coli, Salmonella* sp.) on IBVwas studied by investigating viral shedding.

Where it was recorded that the viral shedding in groups 2 (IB+ *E.coli*) and 4 (IB+ *Salmonella*+ *Escherichia coli*) was higher than in group 1 which given IBV alone. This could occur because the bacteria (*Escherichia coli*) provide the enzymes capable of cleaving the hemagglutinin of viruses (IBV, low pathogenic AIV) enabling them to replicate and spread to a greater extent in that host **(Toshiro *et al.*,1987; Bano *et al.*, 2003).**

Also viral shedding in groups 3 (IB+ *Salmonella*) and 4 (IB+ *Salmonella*+ *Escherichia coli*) was higher than in group 1. This may be due to *Salmonella* infection lead to increase in the level of IL-10 mRNA amounts as well as Treg IL-10 mRNA amounts increased so. IL-10 plays a crucial role in preventing the development of a strong interferon Gamma- driven anti-pathogen response (**Shanmugasundaram *et al.*, 2015)** which may lead to overcoming of virus on immune response of the host and facilitating the replication of virus and thus increasing in viral shedding.

While it was found that viral shedding in group 2 (IB+ *E.coli*) revealed a higher early and prolonged shedding comparable to other infected groups 3 (IB+ *Salmonella*) and 4 (IB+ *Salmonella*+ *Escherichia coli*).

Concerning to the increasing in IBV shedding in group 2 (IB+ *E.coli*) than groups 3 (IB+ *Salmonella*).This may due to as previously discussed, that *E.coli*has a direct effect on IBV activation through providing enzymes responsible for cleaving the thehemagglutinin, so lead to more invasion and replication of virus resulting in more viral shedding in group 2 than group 3.

Meanwhile, the higher IBV shedding in group 2 (IB+ *E.coli*) than in group 4 (IB+ *Salmonella*+ *Escherichia coli*). may be owing to the competitive and antagonizing effect between *Salmonella and Escherichia coli* which come as a result of several causes as in case of increasing in the density of the Enterobacteriaceae facilitates elevated physical contact between *Salmonella* and *E. coli*, resulting in prolific conjugative rates from *Salmonella* to *E. coli* of a conjugative plasmid encoding the bacteriocincolicin 1b. This, process called Horizontal Gene Transfer which occurs between closely related bacteria. Recently it was demonstrated that within enterobacterial blooms, colicin Ib mediated killing of competing *E. coli* confers a distinct growth advantage to *Salmonella* **(Stecher *et al.*, 2012).**

Bacterial pathogens also face a barrage of attack from neutrophils, which have migrated into the intestinal lumen in case of bacterial infection. Approximately 40% of the cytoplasmic nutrient content of neutrophils is composed of a protein named calprotectin **(Fournier and Parkos, 2012)**. Calprotectin has potent antimicrobial activity against many bacterial pathogens including *E. coli* and *Listeriamonocytogenes*, due to its ability to bind and sequester essential metals such as zinc and manganese.

It was observed that in the presence of *Salmonella*, neutrophils are induced to release calprotectin. However, *Salmonella* is able to survive the effects of calprotectin by expressing a high affinity zinc transporter (Znu ABC). This transporter enables the pathogen to grab zinc and provides a growth advantage over the competing bacteria in the inflamed environment of the gastrointestinal tract **(Liu *et al.*, 2012).**

Conclusion

The co-infection between Infectious bronchitis virus, *E.coli* and *Salm-onella* lead to increase in virus replication and virus shedding, than infection with IBV alone so we should give more attention to secondary bacterial infection specially *E.coli* and *Salmonella* and take the suitable preventive measures and precautions against them.

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5/14/2018