

## Investigation Into Prevalence Of *Escherichia coli* Infection And Some Other Diseases In Broiler Farms In Sulaimani District

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### Summary

In regard to broiler diseases in Sulaimani district, different factors like environmental temperature, humidity, wind velocity, the source of hatchery eggs and day-old chicks were studied. The study involved the history, some concurrent diseases, methods of protection against viral diseases, clinical signs, post mortem lesions, isolation and identification methods (cultural and biochemical) and sensitivity test. The 4<sup>th</sup> and 5<sup>th</sup> weeks ages were the exposed ages for respiratory diseases, precisely *Escherichia coli* infection. The temperature factor (high and low) was among the most prominent predisposing factors, since 39% and 30% of *E.coli* isolates obtained through winter and summer seasons successively. The low environmental temperature (5.2°C) accompanied by high humidity rate (72.3 %), caused high number of infections. The high temperature (32.6 °C), with lower rate of humidity (26.2%); has also increased the number of infections. Field investigation including 12 months survey was conducted in the present work. The survey involved 1256 batches from broiler farms, which involved (11,564,385) chicks. A total of 946 (75%) batches showed respiratory signs, including 709 *E.coli* infected batches. Other infections by *Salmonella* and *Proteus* were also discovered. The sensitivity test of 200 *E.coli* isolates revealed that 165, 156 and 118 isolates were sensitive to ciprofloxacin, gentamycin and chloromphenicol respectively. While 182, 181, and 175 isolates were resistant to flumequine, ampicillin and lincomycin consecutively.

التحري عن انتشار أخماج أشريكا القولون و بعض الأمراض الأخرى في حقول دجاج

اللحم في منطقة السليمانية

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### الخلاصة

لم تجري لحد الان اية دراسات عن طبيعة الامراض التنفسية المنتشرة بين قاعات دجاج اللحم في منطقة السليمانية. لذا كان الهدف من هذه الدراسة عمل مسح بياني للامراض التنفسية و دراسة المسببات البكتيرية لهذه الامراض التنفسية ومدى انتشارها والعوامل المساعدة على زيادة نسبتها بصوره

عامة و الخمج بأشريكا القولون بصورة خاصة. تضمن هذا البحث دراسة تأثير العمر، الموسم، العوامل البيئية مثل ( درجة حرارة الجو، الرطوبة وسرعة الرياح) على نسبة انتشار الامراض التنفسية و الخمج بأشريكا القولون ومن خلال النتائج لاحظنا مايلي:-

الاسبوع الرابع ثم يليه الاسبوع الخامس اكثر الاعمار تعرضا للخمج التنفسية بصورة عامة و أشريكا القولون بصورة خاصة. أن عامل الموسم له تأثير على زياده نسبة الخمج بأشريكا القولون ، أذ تم عزل 275 (39%) و 210 (30%) عزلة لأشريكا القولون في فصلي الشتاء و الصيف على التوالي. ان انخفاض درجة الحرارة و ارتفاع نسبة الرطوبة له تأثير على ارتفاع نسبة الخمج بأشريكا القولون حيث تمكن من عزل 105 عزلة عند درجة الحرارة (5.2 درجة مئوية) ورطوبة (72.3%) ، كما وعند ارتفاع درجة الحرارة وانخفاض نسبة الرطوبة عن(40%) وجد ازدياد عدد الاصابات بأشريكا القولون ، أذ تمكن من عزل 89 عزلة لأشريكا القولون عند درجة الحرارة (32.6 درجة مئوية) ورطوبة (26.2%)، اما بالنسبة الى عامل سرعة الرياح ، لم يلاحظ أي تأثير يذكر على زيادة الخمج بأشريكا القولون كون أكثر القاعات شبه مغلقة. خلال هذا البحث أعتمد في التشخيص على ملاحظة العلامات السريرية، والصفات التشريحية والفحوص المختبرية المتمثلة بالزرع والفحوصات البايوكيميائية . واعتمادا على العلامات السريرية لوحظ بأن 946 قاعة (75%) من مجموع 1256 قاعات كانت تعاني من العلامات التنفسية المتمثلة: بفقدان الشهية، العطس، صعوبة التنفس ..... الخ. وقد تمكن من عزل 709 عزلة (75%) لأشريكا القولون. ومن خلال الفحص التشريحي لوحظ الصفات التشريحية التالية: التهاب الاكياس الهوائية الفايبريني، التهاب تامور القلب الفايبريني ، التهاب محفظة الكبد والتهاب الغشاء البريتوني الفايبريني. كما وتبين ايضا خلال الفحص التشريحي أن 110 قاعة من القاعات كانت تعاني من داء الأكريات مع أشريكا القولون، و80 قاعة من القاعات تعاني من مرض الكمبورو IBD مع أشريكا القولون . ومن خلال الفحص المختبري المتمثل بالزرع والفحوصات البايوكيميائية ، تمكن من عزل 15 عزلة *Proteous spp* مع أشريكا القولون، كما وتمكن من عزل 5 عزلة لـ *Avian Salmonella spp* مع أشريكا القولون . اجري ايضا فحص الحساسية لـ 200 عينة من أشريكا القولون المعزولة خلال هذا البحث وتبين بأن 165 و 156 و 118 عينة كانت حساسة لـ chloramphenicol و gentamycin و ciprofloxacin على التوالي، في حين أن 182 ، 181 ، 175 عينة كانت مقاومة لـ flumequine و ampicillin و lincomycin على التوالي.

## Introduction

Respiratory infections in poultry have several causes; the complicated infections usually involve multiple etiologies with viruses, mycoplasmas or other bacteria. Immunosuppressive agents and unfavorable environmental conditions have been also observed. In addition, respiratory reactions induced by routine vaccination programs may themselves play a major role in the development of respiratory diseases (1). Infectious bursal disease in chickens, atmospheric ammonia, dust, and temperature are well known to affect adversely susceptibility to respiratory infection. Most typically flocks that undergo a severe vaccination reaction or the interaction of viral respiratory vaccine viruses with *E.coli*, are the most susceptible to common respiratory disease (2, 3). *E.coli* respiratory infection was first described in chickens in 1894. Since then, many reports on colibacillosis in poultry have been reported (4). Some avian pathogenic *E.coli* strains are belong to the same clones of pathogenic *E.coli* isolated from human intestinal infections (5). Normal, healthy birds with intact defenses are remarkably resistant to naturally *E.coli* exposure, but Infection occurs when skin or mucosal barriers are compromised or birds are exposed to abnormal stress (6, 7). Moderate stress was reported to increase resistance, possibly as a result of the development of immunity following contact of organisms with the immune system, or as a result of exercising defense mechanisms and maintaining them in a state of readiness (8). Clinical disease is reported most often in chickens, turkeys, ducks and pheasants (9, 10, and 11). *E.coli* are normal inhabitants of the lower digestive tract of many avian species, both pathogenic and non Pathogenic *E.coli* isolates can be recovered at pharynx , trachea, skin and feathers from healthy birds (12), Vertical transmission of *E.coli* from breeders, via contaminated shell with faecal materials during hatching, or in ova, as a result of salpingitis may occur(13). Birds are frequently infected by inhalation of dust which may contain *E.coli* (12). Airsacculitis is the most important disease syndrome associated with avian pathogenic *E.coli*. The respiratory tract complex is most often observed in birds of 4 to 9 weeks of age and may result in extensive economic losses with up to 20% mortality, reduced growth and increased condemnation rate at the abattoir (10). Depression, fever, drops in food consumption, listless and standing about dejectedly with ruffled feathers are clinical signs in this infection. Morbidity and mortality are variable and losses are usually less than 5% of the group, but morbidity can be over 50% (10). Survivors show signs of unthriftiness (13). Airsacs become thickened, containing caseous, cheesy material. The infection may cause accumulation of fibrin on the surface of the visceral organs, A septicemic carcass may show dark and congested viscera (10), in younger chicks the ceca may be filled with caseous material (14). The most characteristic lesions are green liver and in

some cases multiple pale foci in the liver have been described, also marked splenomegaly, and congested muscles (11, 15).

The present work was designed to determine the rate and effect of respiratory diseases in broilers of Sulaimani district, and to determine the etiology of some respiratory diseases. For this purpose clinical observations together with postmortem, cultural, biological and sensitivity tests were carried out. The investigation included studying environmental factors, management, presence of other diseases, sources of infection and their relations with the virulence of the infection.

### **Materials And Methods**

The present investigation involved broiler poultry farms at different ages, around Sulaimani city and during a period of 12 months (1/10/2001-30/9/2002). Data were collected from 1256 broiler houses; samples were collected randomly almost from all the infected houses. The total of 946 samples were examined (Table: 1). The sources of one-day old chicks were different, including Arbil, Duhok, Iran, and Sulaimani hatcheries. Chicks were also obtained from the central poultry laboratory /veterinary hospital /Sulaimani city. The clinical signs and post mortem lesions were recorded. Samples from heart-blood were inoculated in nutrient broth or MacConky broth immediately and incubated for 18-24 hours at 37°C. Gram stain technique was used for staining bacterial growth. Samples from gram-negative bacteria were transferred into MacConky agar and incubated for 24 hours at 37 °C, for studying colonies characteristics and lactose sugar fermentation ability.

In order to determine the biochemical properties of the organisms, each time 3-5 colonies were taken ,and cultured on the following media :Indol media, urea media, Kligler's iron agar, gelatin media, Simmon's citrate media, methyl red, Vogus- Proskour and motility test was also performed. After isolation, identification and purification of each *E.coli* isolate, it was transferred to heart – brain infusion agar and kept at 4°C (16).

Acriflavin agglutination test was used according to the method described (17). Loop full of the isolated *E.coli* was taken from nutrient agar surface into glass slide, mixed it gently with a large drop of the acriflavin solution (1:500) and the positive result (agglutination) was recorded. Sensitivity test of *E. coli* isolates from diseased cases was done according to the technique reported by Bauer and Kirby (18). The age and environmental factors on the broiler susceptibility to respiratory diseases generally and *E.coli* precisely were also studied. The statistical analysis was done, using X<sup>2</sup> according to a methods described by Snedcor and Cochran (19).

## Results

From a total of 1256 broiler houses, 946 houses were suffering from respiratory diseases with clear clinical signs like: gasping, breathing difficulties, listlessness, reduced food consumption, ruffled feathers, among them 45 houses were suffering from swelling head. Other diseases like Infectious bursal disease, Newcastle disease, Inclusion body hepatitis, coccidiosis and nutritional deficiency were observed from 242 houses. The results are shown in Table: 1 and Fig.1.

Post mortem lesions were recorded like follows:

141 (11%) only airsacculitis, 252 (20%) airsacculitis with peritonitis, perihepatitis, pericarditis, 121 (9%) airsacculitis and pericarditis, 83 (7%) airsacculitis and peritonitis, 110 (9%) airsacculitis and coccidiosis, 80 (6%) airsacculitis and infectious bursal diseases, 121 (10%) coccidiosis, 65 ( 5% ) only infectious bursal diseases, 2 (0.15%) Newcastle disease, 3 (0.23%) inclusion body hepatitis, 51 (4.06%) nutritional deficiency, 68 (5.41%) without pathological lesion.

Among samples collected from 946 poultry houses, 709 *E. coli* isolates were identified. Mixed cultures which consisted of *Proteus spp.*, or *Salmonella spp.* were also included. The results of biochemical testes that performed for identification of *E.coli*, *Proteus spp.* and *Salmonella spp.*, were recorded. All isolated avian *E.coli* (709 isolates), were unable to agglutinate acriflavin solution at 1:500 concentrations. The effect of age on the broiler susceptibility to respiratory diseases generally and *E. coli* infection particularly was also determined. The pike of respiratory infection and isolation of *E.coli* were at ages of fourth and fifth weeks respectively. Regarding humidity, temperature and wind velocity, data were obtained from the meteorological center of Suliamani Governorate. The Statistical analysis and seasonal effects on respiratory cases generally and *E.coli* infection are precisely shown in Table 2 and Fig. 3. A total of 200 isolates from colisepticemia confirmed cases were obtained in profuse culture from both heart and liver tissues. The antimicrobial resistance of the isolates to different antibacterial agents was determined by the standard disc diffusion method in Muller-Hinton agar with disc provided by Razi center. The results revealed that 165, 156 and 118 isolates were sensitive to ciprofloxacin, gentamycin and chloromphenicol successively. While 182, 181, and 175 isolates were resistant to flumequine, ampicillin and lincomycin consecutively.

## Discussion

According to the results that obtained from the present investigation, a great annually economical loss could be predicted from Sulaimani district, due to *E.coli* respiratory infection. The precisely estimated loss cannot be assumed, since the obtained data was only from the farms that were submitted to our investigation. However, *E.coli* is well known as one of the devastating poultry problems for poultry industry (12). From 1256 broiler poultry houses, involved in this investigation, 946 houses have shown respiratory signs (Table 2). The signs were gasping, nasal discharge, sneezing, listlessness, reduced food consumption and ruffled feathers. These signs had been reported also by others (13, 15, and 20). Swelling head either alone or combined with other clinical signs were also observed from 45 (5%) houses from total 946 infected houses. However swelling head has been reported by several workers (5, 21, and 22). The post mortem lesions observed in the present work were fibrinous airsacculitis, pericarditis, perihepatitis, peritonitis and enteritis. Such lesions were also reported previously (11).

Isolation and identification of *E.coli* and other bacteria is shown in Fig.1. Seventy five percent (75%) of isolates were identified as *E.coli*, (Table 2). The *E.coli* isolates that obtained from infected cases were identified according to the standard laboratories tests. These tests have been recommended by many authors including (23, 24).

An agglutination test with acrifavin dyes 1:500 was used for differentiation between the smooth and rough pathogenic strains. The negative results showed by the 709 *E.coli* isolates (100%), have supported the identification, similar work was done also (15,17).

Although reference is not available, regarding effect of *Proteus spp.*, in avian respiratory diseases, two percent (2%) of the total isolates were *Proteus spp.*, and (1%) of isolates were *Salmonella spp.*, which is well known as one of the causative agents of respiratory diseases in chickens (25). The motility test for isolated salmonella revealed no motility, so the isolates may be either *Sal.pullorum* or *Sal.gallinarum* (26). This finding for sure needs more investigation in future.

The present work involved studying the effect of age factor on the *E.coli* infection and other respiratory diseases. The results showed that 4<sup>th</sup> and 5<sup>th</sup> week's age were more susceptible ages for such diseases. This factor reported also by other workers (13, 27).

The effects of environmental factors like temperature, moisture and wind velocity (Table 2) were also studied in this work. The summer peak of respiratory infection was in July and August, when the temperature range from 31.2 - 32.6°C, this can be explained by considering heat as a stress factor (11). Since increased temperatures cause anorexia, difficult respiration, drinking more

water, diarrhea, particularly when the moisture rate is high, it produces wet bedding, ammonia liberation, deceleration of the upper respiratory tract. Wet bedding may also facilitate spread of coccidiosis, which is a recurrent infection or predisposing factor for *E.coli* infection (11). When the high temperature is accompanied by a low moisture rate, this may lead to desiccation and increased dust production which is also predisposing factor for *E.coli* infection (1).

The low temperature increases rate of *E.coli* infection (Fig. 4). Cold air draught and chilling are usually considered as stress and predisposing factors (1, 11).

In the present work the winter peak of respiratory infection was between December and January when the climate temperature was ranged between 5.2 - 8.7°C (Table 2). It is worthy to point out that the construction materials, designing and directions of all poultry houses in the area had been implemented regardless of both essential requirements, of the broilers and isolation value of the buildings. So the environment weather affect adversely on the temperature inside the poultry houses and also on the health and production of the poultry. Moreover the heating and cooling systems, fans and other equipments in the local broiler's houses are neither enough nor efficient. The results of present work have also showed the influences of environmental moisture and wind (Fig.4 and Table 2).

However, the data regarding moisture rate and wind velocity were obtained from meteorological center of Sulaimani Governorate which may not be convenient with all the broiler's houses that involved in the present work, though it would be more convenient if these data were collected in nearby places, since the houses that involved had been scattered over a wide area in Sulaimani district. The statistical analysis regarding the prevalence of infections according to the different seasons, are shown in Fig. 2. There were no significant statistical variation ( $P > 0.1$ ), since there are many viral, bacterial, mycoplasma and fungal, also environmental factors which produce respiratory diseases, regardless of seasons. Concerning *E.coli* infection (Table 2, Fig. 1), there was a significant statistical difference ( $P < 0.01$ ) according to different seasons of the year.

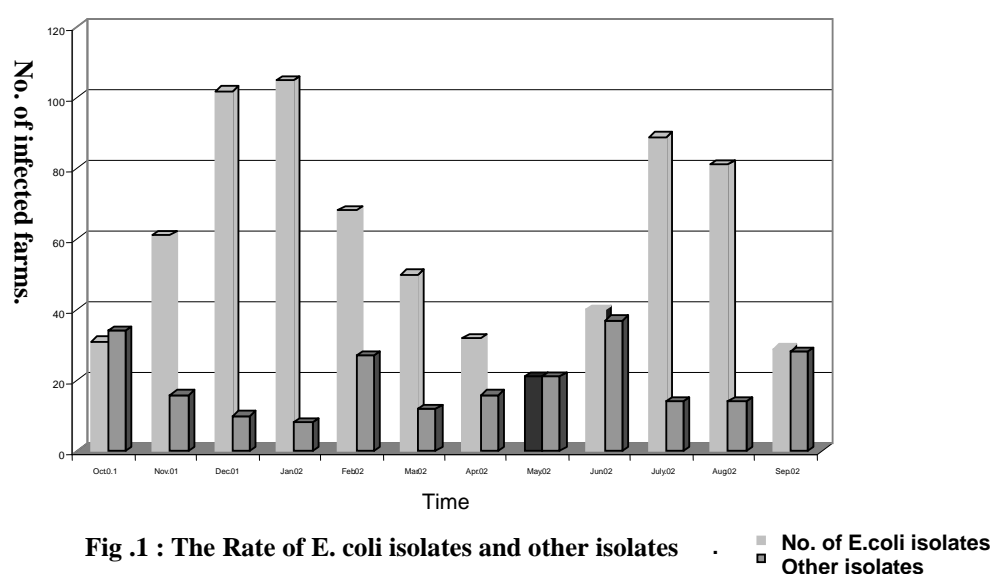
**Table 1: Cases which diagnosed according to the clinical signs and post mortem lesions throughout 12 month period of Investigation.**

No.	Months	Houses infected with airsaccu-litiss	Houses infected with airsaccu-litiss & coccidiosis.	Houses infected with airsaccu-litiss & IBD.	Houses infected with coccidiosis	Houses infected with IBD	Houses infected with IBH	Houses infected with ND	Houses with nutritional def.	Houses with out any patho. changes	Total
1	Oct. 01	54	4	7	7	5	-	-	15	3	95
2	Nov. 01	54	8	15	9	4	-	2	-	-	92
3	Dec. 01	105	7	-	23	10	3	-	7	5	160
4	Jan. 02	83	29	1	23	2	-	-	5	10	153
5	Feb.02	78	15	2	11	-	-	-	4	5	115
6	Mar. 02	39	15	8	9	4	-	-	2	4	81
7	Apr. 02	41	5	2	7	-	-	-	-	8	63
8	May 02	38	4	-	7	2	-	-	-	8	59
9	June 02	63	4	10	3	5	-	-	3	9	97
10	July 02	91	-	12	-	15	-	-	12	5	135
11	Aug. 02	72	4	19	12	11	-	-	-	3	121
12	Sep.2002	38	15	4	10	7	-	-	3	8	85
	<b>Total</b>	<b>756</b>	<b>110</b>	<b>80</b>	<b>121</b>	<b>65</b>	<b>3</b>	<b>2</b>	<b>51</b>	<b>68</b>	<b>1256</b>



**Table 2: The relations of environmental factors with the numbers of respiratory infected cases throughout the 12 months period.**

No.	Months	Rate of humidity %	Temperature / Average °C	Wind velocity W/C	Number of respiratory cases	Number of <i>Escherichia coli</i> isolates	Number of infected cases (Total)
1	Oct.2001	34.3	22.4	1.6	65	31	95
2	Nov.2001	49	13.1	0.9	77	61	92
3	Dec.2001	77.9	8.7	0.5	112	102	160
4	Jan.2002	72.3	5.2	1.2	113	105	153
5	Feb.2002	56	9	1.4	95	68	115
6	Mar.2002	49.5	13.6	2.2	62	50	81
7	Apr.2001	65.3	14.9	1.1	48	32	63
8	May.2002	38.5	22.6	1.5	42	21	59
9	June.2002	27.6	29.1	2.2	77	40	97
10	July.2002	26.2	32.6	2.3	103	89	135
11	Aug.2002	29.7	31.2	1.4	95	81	121
12	Sep.2002	29.6	28.1	1.0	57	29	85
					<b>946</b>	<b>709</b>	<b>1256</b>



**Fig .1 : The Rate of E. coli isolates and other isolates** . ■ No. of E.coli isolates ■ Other isolates

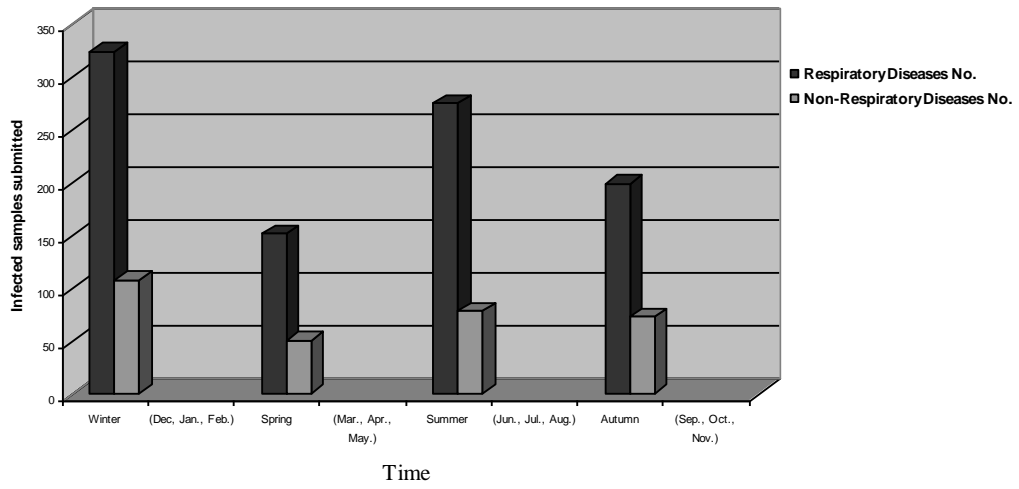


Fig.2: The samples of clinically infected cases (Respiratory and non respiratory diseases) throughout different seasons.

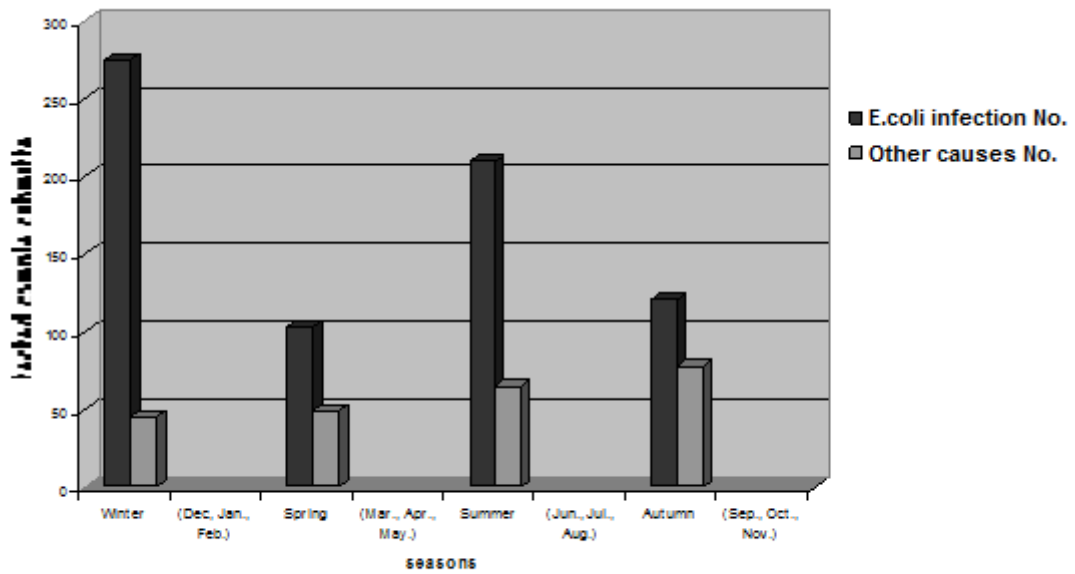


Fig. 3: The samples of clinically infected cases (*E.coli* infection and other causes ) throughout different seasons .

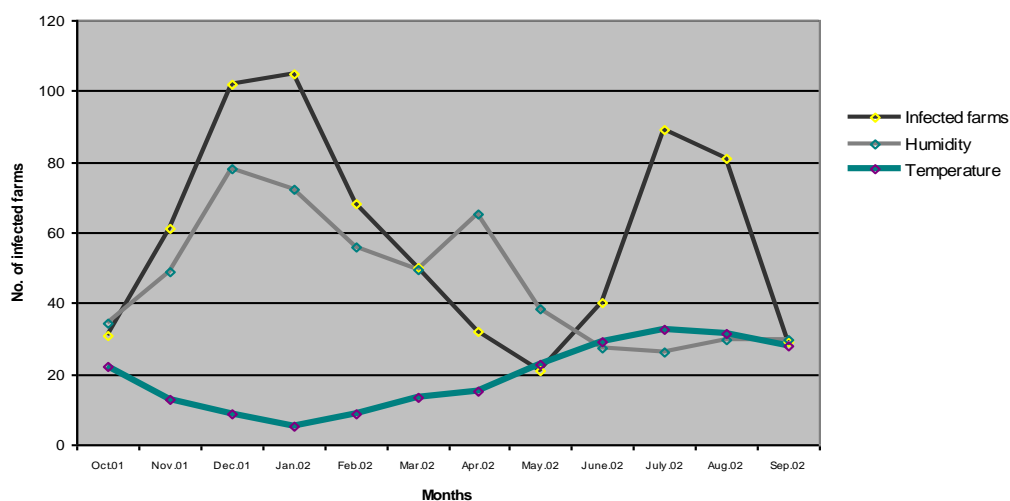


Fig. 4: The effect of Temperature °C and Humidity % on *E.coli* infection.

Other factors which influence the exacerbation of *E.coli* infection in the local broiler's house are also observed. These extra factors include introducing larger number of chicks per one m<sup>2</sup> by the poultry men i.e. more than the standard or allowable number, hoping more profit or as spare, to compensate the expected mortality. It is quite clear that extra number of chicken produce a great stress, wet litter, bad ventilation, retard of growth and more susceptibility to infections particularly *E.coli* infection.

Application of different types of vaccines, by local unskillful poultry men, in a short period of 45 or 50 days, and from different firms or origins, This is another stress factor which increase susceptibility of chicken to *E.coli* infection (28).

The use of antibacterial drugs by poultry men, regardless of veterinary advice or scientific principles, the drug marketing or drug handling without proper supervision by Governmental specialist authorities may produce a great hazard for public health and poultry production sector, so it should be a special legislation for control and monitoring such kind of drug application.

Another concurrent factor which exacerbates *E.coli* infection is coccidiosis, which was observed by many workers (7). In this work, the above observation has demonstrated in Table 1. Unbalanced ration, malnutrition, and vitamins deficiency are also predisposing factors for *E.coli* (11). These factors particularly appear when the ration is not provided by a reputable feed factory, like that which practiced locally i.e. the ration ingredients are mixed by the broilers owner, who has no enough background to produce an efficient and balanced ration. So adding or neglecting some essential nutrients or replace some ingredients either in purpose or because of economical factors e.g.

addition of cooking oil as a source of energy, instead of the maize, and when store for several days this may cause nutritional deficiency especially (vitamin E) which leads to reduced immunity and provide change to opportunistic microorganism like *E.coli* to play role and produce infection (29, 30, 31).

The sensitivity tests were performed to 200 *E.coli* isolates obtained from infected cases. There was a wide range of variation regarding sensitivity or resistance to different applicable antibiotics. This may be explained by variable source of day- old chicks, since almost Arbile then Dohuk and Iran are the main suppliers of day- old chicks or due to a randomly application of antibiotics, or due to lack of hygienic measures and a low standard of poultry management in the area.

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