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SOME STUDIES ON HAFNIA ALVEI IN CHICKENS

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ABESTRACT

This study was aimed to study the epidemiology and the possible ways of treatment of Hafnia alvei (H. alvei) as an enteric pathogen in laying hens during the period from 2007-2010. For this purpose, bacteriological examination of samples collected from 500 laying hens from different localities in Gharbia Province suffering from drop in egg production, decreased hatchability, inappetance, diarrhea, opisthotonus and mortalities revealed isolation of 18 isolates of H. alvei with an incidence of 3.6%. Phage-typing of the isolated strains using Hafnia specific bacteriophage produce clear plaques of lysis with a zone 1-2 mm in diameter confirmed the organism. Antibiogramme of the isolated H. alvei strains revealed high sensitivity to Fosfomycin+tylosin, cephotaxime, Colistin sulphate, Gentamycin, Norfloxacin, Spectinomycin and Ciprofloxacin but were resistant to Amoxicillin, Clindamycin , Enrofloxacin , Cephradine, Kanamycin, Lincomycin, Sulphamethozole trimethoprim, Streptomycin, Spiramycin and Penicillin G. Experimental infection of 38-week-old laying hens with isolated strains of H. alvei and treatment trials using Fosfomycin+tylosin and Garlicin were carried out. Clinical signs, postmortem lesions with re-isolation of the infected strains were discussed in details. Histopathological examination of different organs from experimentally infected hens revealed less sever lesions after treatment with Fosfomycin+tylosin.

Keywords: *Hafnia. alvei* infection, laying hens, bacteriology, histopathology, and treatment.

INTRODUCTION

Hafnia alvei is a relatively recent discovered bacterium of the Family *Enterobacteriaceae* responsible for enteric disease in laying hens, and has been identified as one of emerging enteric bacterial pathogens. *Hafnia alvei* is a motile Gram-negative factitively anaerobic bacillus classified as a causative agent of intestinal disorders, including gastroenteritis, septicemia and decreased egg production in laying hens ***(Real et al., 1997).***

Hafnia alvei has been reported to cause large outbreak in 12-week-old turkey poults in Italy suffered from anorexia, depression, ruffled feather, diarrhea and mortalities 20.7% with enteritis and splenomegally ***(Proietti et al., 2004).***

Hafnia alvei can transmit horizontally as well as vertically in naturally and experimentally infected broiler breeders ***(El-Gohary and Youseif, 2002).***

This work was aimed to investigate the incidence *Hafnia alvei* in laying hens in Gharbia province, to identify the isolated strains of *Hafnia alvei* morphologically, biochemically and by phage-typing, to study the pathogenicity of some isolated strains and their sensitivity to various antibacterial agents in-vitro and in-vivo as well as histopathological examination of organs in experimentally infected hens.

MATERIALS AND METHODS

Samples collection:

Specimens were collected from 500 diseased, freshly dead and slaughtered laying hens suffering from gastroenteritis obtained from different localities in El- Gharbia province from 2007-2010. The history of tested layer chicken flocks include number of birds in each flock, breed, clinical signs, post-mortem lesions as well as mortality rate were recorded and presented in Table (1).

specimens collection:

specimens from liver, spleen, kidney, small intestine, heart, lung, bone marrow and ovary, were collected and subjected to bacteriological examination. Sterile cotton swabs inserted into the cloaca also used for bacteriological isolation.

bacteriological isolation:

A loopful from collected organs or cloacal swabs were streaked on Nutrient agar, Brain heart infusion agar, Trypticase soya agar, MacConkey's agar Salmonella-shigella (S.S) agar. ***(Sakazaki, 2005)***. The suspected colonies were picked up, purified and identified morphologically, and biochemically. *H. alvie* suspected colonies were identified using phage 1672 (ATCC), a *Hafnia* specific bacteriophage show specific plaques of lysis with *Hafnia* strains according to the method described by ***Guine´e and Valkenburg, (1968)***.

Antibiogramme:

The antibiogramme of isolated *Hafnia. strains* was investigated against 18 antimicrobial agents using the disc diffusion technique according to ***Cruick-shank et al., 1975***.

Experimental infection:

Fourty laying hens 36-week-old were obtained from layer farms were proved to be free from *Hafnia* infection were floor reared in eight clean and disinfected partitions, Fed finished pellet ration without antibiotics (Table 2).

Histopathological studies:

Samples from liver, spleen, lung, kidney, intestine and ovaries were fixed in 10% neutral formaline. The washed soft tissues were dehydrated in different concentration of alcohol, cleared in xylol and embedded in paraffin. Sections of 5 micrometer were then cut and stained with H&E stain according ***Lillie (1984).***

Table (1): History of examined 50 layer farms in Garbia province.

Code No	Districts	Breed	House capacity No.	Age / weeks	Clinical signs	P.M lesions	Mortality % per week	Drop in egg prod. % per week	Vaccination Schedule
1	Berma	Balady	10.000	30	Diarrhea, drop in egg production	Oophoritis and pericarditis	2.9	12.3	IB-ND-EDS
2	Berma	Balady	10.000	30	Inappetance and decreased hatchability	Pneumonia, Air saculitis and oophoritis	5. 3	11.0	IB-ND-EDS
3	Berma	Balady	10.000	30	Decreased egg production	Splenomegally, thickening of intestinal wall	2.7	15.6	IB-ND-EDS
4	Berma	Balady	15.000	42	Decreased egg production	Oophoritis and pericarditis	4.3	13.6	IB-ND-EDS
5	Berma	Balady	20.000	55	Diarrhea	Enteritis	4.5	8.7	IB-ND
6	Berma	Balady	25.00	47	Mortalities	Necrotic foci on liver	3.4	16.4	IB-ND-EDS
7	Berma	Bovans white	25.000	27	Decreased egg production	Catarrhal exudates in intestine	2.5	16.1	IB-ND
8	Berma	Bovans white	25.000	43	Mortalities and decreased egg production	Oophoritis	3.9	19.2	IB-ND-EDS
9	Talbnt	Bovans white	10.000	39	Mortalities, Opisthotonus and decreased egg production	Necrotic foci on liver	5	12.5	IB-ND-EDS
10	Talbnt	Bovans white	10.000	39	Mortalities, diarrhea and decrease hatchability	Enteritis	6.7	12.7	IB-ND-EDS
11	Talbnt	Bovans white	15.000	42	Arthritis and diarrhea	Enteritis and oophoritis	5.9	9.7	IB-ND
12	Talbnt	Bovans brown	20.000	40	Diarrhea,drop in egg production	Abnormal kidney	2.5	13.3	IB-ND-EDS
13	Talbnt	Bovans brown	20.000	40	Respiratoey signs and decreased egg production	Trachitis	2.8	11.4	IB-ND
14	Talbnt	Bovans brown	10.000	33	Diarrhea	Enteritis	4.7	9.3	IB-ND-EDS
15	Talbnt	Bovans brown	5.000	36	Decreased egg production	Catarrhal exudates in intestine	5.5	12.2	IB-ND
16	Sobtas	Balady	10.000	44	Diarrhea and decrease in egg production	Oophoritis	3.3	14.7	IB-ND-EDS
17	Sobtas	Hisex brown	12.000	54	Inappetance	Necrotic foci on liver	2	15.9	IB-ND
18	Sobtas	Balady	10.000	36	Decreased egg production	Spleenomegally and oophoritis	3.8	14.2	IB-ND-EDS
19	Kfr el-shegh selem	Bovans white	10.000	35	Decreased egg production	Oophoritis,pericaqrditis, and pneumonia	4.1	12.3	IB-ND-EDS
20	Kfr el-shegh selem	Balady	50.000	50	Decreased egg production	Catarrhal exudates in intestine	3.1	11.0	IB-ND
21	Kfr el-shegh selem	Balady	50.000	33	Diarrhea	-	2.9	13.4	IB-ND-EDS
22	Berma	Bovans white	12.00	32	Diarrhea	Catarrhal exudates in intestine	2.2	14.6	IB-ND-EDS
23	Talbnt	Hisex brown	15.00	33	Decreased egg production	Necrotic foci on liver	4.6	16.5	IB-ND

Code No	Districts	Breed	House capacity No.	Age / weeks	Clinical signs	P.M lesions	Mortality % per week	Drop in egg prod. % per week	Vaccination Schedule
24	Talbnt	Balady	5.000	39	- -	Necrotic foci on liver	4.9	10.4	IB-ND-EDS
25	Talbnt	Balady	5.000	40	Decreased egg production	Oophoritis,splenomegally, enteritis and pericarditis,	5.1	13.8	IB-ND-EDS
26	Wqa	Balady	10.000	40	Decreased egg production and hatchability	-	3.9	12.9	IB-ND-EDS
27	Tanta	Balady	5.000	42	Decreased egg production	Spleenomegaly and Necrotic foci on liver	4.1	12.4	IB-ND
28	Tanta	Balady	30.000	55	-	Necrotic foci on liver	3.2	11.8	IB-ND
29	Tanta	Balady	30.000	50	Diaarhea	Abnormal friable yellowish liver	4.7	13.5	IB-ND-EDS
30	Berma	Balady	5.000	41	Decreased egg production	Oophoritis	5.3	11.3	IB-ND
31	Berma	Bovans brown	20.000	40	Respiratory signs and diaarhea and decreased egg production	Necrotic foci on liver,splenomegally and oophoritis	6	17.1	IB-ND
32	Met hway	Balady	15.000	37	Diarrhea	Enteritis	4.9	12.6	IB-ND-EDS
33	Met hway	Balady	15.000	39	Diarrhea	-	4.1	12.2	IB-ND
34	Tanta	Bovans brown	20.000	40	Diarrhea	Necrotic foci on liver	5.4	10.1	IB-ND
35	Berma	Balady	15.000	33	-	Enteritis	2.9	12.6	IB-ND-EDS
36	Berma	Balady	15.000	37	Decreased egg poroduction	Oophoritis	4.3	13	IB-ND
37	Fesha slem	Hisex brown	10.000	42	Decreased egg poroduction and diarrhea	Dehydrated carcuss	5.1	14.1	IB-ND-EDS
38	Shony	Bovins white	20.000	43	Inappetance,and decreased hatchability	Oophoritis	4.2	18.5	IB-ND-EDS
39	Shony	Bovins white	20.000	51	Respiratory signs	Pneumonia and pericarditis	6.1	12	IB-ND-EDS
40	Berma	Hisex brown	15.000	44	Mortalities and diarrhea	-	3.7	11.1	IB-ND
41	Met hway	Balady	5.000	53	Opisthotonus	Oophoritis,pericaqrditis, and pneumonia	5.3	10.7	IB-ND-EDS
42	Berma	Balady	10.000	39	Respiratory signs and diaarhea	-	2.7	14.1	IB-ND
43	Berma	Bovans brown	20.000	59	-	Necrotic foci on liver	2.6	12.4	IB-ND-EDS
44	Berma	Bovans brown	20.000	55	-	-	2.2	13	IB-ND-EDS
45	Berma	Balady	12.000	40	Opisthotonus	Oophoritis	5.4	12.2	IB-ND-EDS
46	Berma	Balady	12.000	57	-	Enteritis	4.6	11	IB-ND
47	Berma	Balady	12.000	45	Diarrhea	Enteritis	3.9	.214	IB-ND
48	Berma	Balady	12.000	42	Decreased egg production	Oophoritis	5.9	16.3	IB-ND
49	Berma	Balady	25.000	30	Respiratory signs and diaarhea	Pneumonia and pericarditis	4.9	10	IB-ND
50	Berma	Balady	10.000	33	Opisthotonus	Oophoritis, pericaqrditis, and pneumonia	3.6	9.7	IB-ND-EDS

Table (2): Experimental design for infection with *H. alvei* and treatment of layers.

Group codes	Infection and Treatment	Breed	No.of Birds	Inoculation and dose
1	Non infected-Non treated	Balady	5	Inoculated orally with 1 ml of sterile saline (Blank control).
2	Non infected-treated with Fosbac	Balady	5	Inoculated orally with 1 ml of sterile saline then give fosbac 160 mg/ kg b.wt for 5 days on drinking water (Controle –ve).
3	Non-infected treated with garlicin	Balady	5	Inoculated orally with 1 ml of sterile saline then give garlicin 1 gm / 1iter water for 5-7 days (Controle –ve).
4	Infected treated with Fosbac	Balady	5	Inoculated orally with a dose of $1*10^6$ then treatead with fosbac 160 mg/kg b.wt for 5 days after 3 weeks.
5	Infected treated with garlicin	Balady	5	Inoculated orally with a dose of $1*10^6$ then treated with garlicin 1 gm / 1iter water for 5-7 days after 3 weeks.
6	Infected treated with both fosbac and garlicin	Balady	5	Inoculated orally with a dose of $1*10^6$ then treated with both drugs after 3 weeks.
7	Infected non treated	Balady	5	Inoculated orally with a dose of $1*10^6$and non treated (Controle +ve).
8	Non-infected treated with both fosbac and garlicin	Balady	5	Inoculated orally with 1 ml of sterile saline (Controle –ve).

RESULTS AND DISCUSSION

Examined birds were suffering from loss of appetite, ruffled feathers, diarrhea, opisthotonus and sudden mortalities of 2-6.7%, lower in egg production (8.7-19.2%) and lower hatchability (5-9%), with decreased egg size.

The main gross lesions of dead and scarified birds revealed enlarged liver with numerous randomly scattered whitish-yellow foci, 2-3 mm in diameter, oophoritis, pericarditis, egg peritonitis, pneumonia, Splenomegally and a diffuse thickening of the intestinal wall with catarrhal exudates on the mucosal surface. Similar results were reported by ***Real et al., (1997), El-Gohary and Youseif (2002) and Proietti et al., (2004).***

H. alvei isolation from the internal organs of infected commercial layers revealed isolation of Gram negative, facalatitvelly anaerobic bacilli. Out of 500 diseased laying hens 18 *H. alvei* isolates (3.6%) were

isolated Table (3). The highest incidence of isolation was obtained from liver(1.4%), followed by the ovary (0.8%), heart, lung and spleen (0.66%), intestine (0.4%), kidney and bone marrow (0.2%) Table (4). Similar findings were obtained by ***El-Gohary and Youseif (2002).***

API 20 E strip system and *Hafnia* specific bacteriophage1672 provided the final reliable identification and differentiation of *H. alvei* from other bacteria, which cause similar clinicopathological effects. Similar results were recorded by ***Real et al., 1997.***

The results of in-vitro antibiotic sensitivity test revealed that all tested isolates of *H. alvei* were highly sensitive to fosfomycin+tylosin, cephotaxime, Colistin sulphate, Gentamycin, Norfloxacin, Spectinomycin, Ciprofloxacin but were resistant to Amoxicillin, Clindamycin, Enrofloxacin, cephradine, Kanamycin, Lincomycin, Sulphamethozole trimethoprim, Streptomycin, Spiramycin, Penicillin G. (Table 5). These result were similar to the results obtained by ***Proietti et al., (2004), stock et al., (2005) and Liu et al., (2007).***

The experimental infection of 36-week-old laying hens with $1x10^6$ CFU orally revealed that *H. alvei* isolates was capable of inducing 7.14% mortalities, drop in egg production and diarrhea (Fig 1) A, B, C & D with successful re-isolation of the organism post infection. Similar observations were obtained by ***Real et al., 1997.***

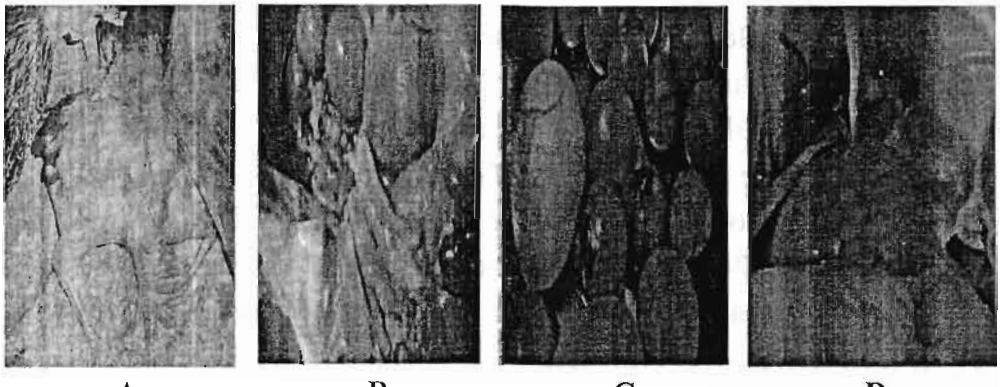
The result of isolation of *H. alvei* from 48 laid eggs revealed positive result in 24 eggs (50%) indicated vertical transmission.

Treatment trials was based on the results of in-vitro sensitivity test of the isolated *H. alvei* organisms.

The clinical signs and postmortem lesions disappeared in experimentally infected hens and treated with Fosfomycin+Tylosin and with Garlicin in drinking water for 7 consecutive days when compaired with infected untreated control group (Table 6). Similar results were obtained by ***El-Gohary and Youseif (2002).***

Histopathological examination of experimentally infected hens revealed lesions of internal organs as liver, showing congestion of portal vein, portal area expanded by large numbers of lymphocytic and heterophilic infiltration, vacuolar and hydropic degeneration of hepatocytes and pyknosis of nuclei of individual hepatocytes **(Fig 2**, A). Kidney revealed hypertrophy and hypercellularity of some glomeruli, coagulative necrosis of lining epithelium of renal tubules and multiple focal areas of hemorrhages in renal cortex **(Fig 2**, B). Lung revealed sever congestion of blood vessels and leucocytic infiltration of bronchi. Intestine showed desqumation of the lining epithelium and leucocytic infiltration of lamina propria **(Fig 2**, C). Spleen showed lymphoid depletion, while ovareies had eosinophilic infiltration in wall of blood vessels and there were focal area of coagulative necrosis **(Fig 2**, D). Similar finding were obtained by ***Real et al., (1997).***

Histopathological examination of examined organs of *H. alvei* infected hens at 15 days post treatment with Fosfomycin+Tylosin revealed less sever lesions **(Fig 3, A&B)**.



A B C D

Fig (1): Experimentally infected chickens with *H. alvei* showing the clinicopathological pictures of the disease(A), Oophoritis (B and C), Abnormal liver with randome scattered whitish yellow foci throughout the parenchyma, pneumonia and air saculitis (D).

Table (3): Results of *H. alvei* isolation from different locations in Gharbia

Farm No.	M %	R	Farm No.	M %	R	Farm No.	M %	R	Farm No.	M %	R	Farm No.	M %	R
1	2.9	-ve	11	5.9	-ve	21	2.9	+ve	31	6	+ve	41	5.3	-ve
2	5.3	-ve	12	2.5	+ve	22	2.2	-ve	32	4.9	+ve	42	2.7	+ve
3	2.7	+ve	13	2.8	-ve	23	4.6	-ve	33	4.1	-ve	43	2.6	-ve
4	4.3	-ve	14	4.7	-ve	24	4.9	-ve	34	5.4	+ve	44	2.2	-ve
5	4.5	-ve	15	5.5	+ve	25	5.1	+ve	35	2.9	-ve	45	5.4	-ve
6	3.4	+ve	16	3.3	-ve	26	3.9	+ve	36	4.3	-ve	46	4.6	-ve
7	2.5	+ve	17	2	-ve	27	4.1	-ve	37	5.1	-ve	47	3.9	+ve
8	3.9	-ve	18	3.8	-ve	28	3.2	+ve	38	4.2	-ve	48	5.9	-ve
9	5	+ve	19	4.1	+ve	29	4.7	-ve	39	6.1	+ve	49	4.9	-ve
10	6.7	-ve	20	3.1	-ve	30	5.3	+ve	40	3.7	-ve	50	3.6	-ve

M%= mortality % R= result of isolation

Table (4): Prevalence of *H. alvei* isolates in various organs of laying hens in Gharbia Province.

Organs	No. of examined organs	No. of isolates	Percentage of isolation (%)
Liver	500	7	1.4
Lung	150	1	0.66
Heart	150	1	0.66
Ovary	500	4	0.8
Kidney	500	1	0.2
Bone marrow	500	1	0.2
Joint	50	0	0
Intestine	500	2	0.4
Spleen	150	1	0.66
Total	3000	18	0.6

Table (5): Results of in-vitro sensitivity test of *H. alvei* isolates against different 18 antibicrobial agents using.

Serial No.	Antibacterial agent	Potancy of disc (µg)	Standard sensitivity zone mm			Zone of inhibition mm	S/R
			R	I	S		
1	Amoxicillin (AML)	10	13	14-17	18	0.4	R
2	Cephotaxime (CTX)	30	14	15-22	23	31	S
3	Clindamycin (DA)	2	14	15-16	17	0	R
4	Colistin sulphate (CT)	25	10	12-13	14	14	S
5	Doxycycline (DO)	30	12	13-15	16	10	R
6	Ciprofloxacin(Cip)	5	15	16-20	21	27	S
7	Cephradine (CE)	30	15	16-20	21	14	R
8	Enrofloxacin (ENR)	5	16	17-20	21	16	R
9	Fosfomycin plus Tylosin (F+T)	-	-	-	-	33	S
10	Gentamycin (CN)	10	12	13-14	15	21	S
11	Kanamycin(K)	30	13	14-17	18	0	R
12	Lincomycin (My)	10	14	15-20	21	0	R
13	Norfloxacin(NOR)	10	12	13-16	17	28	S
14	Sulphamethozole trimethoprim (SXT)	25	10	11-15	16	0	R
15	Streptomycin (S)	10	11	12-14	15	0.8	R
16	Spectinomycin (SH)	100	12	13-14	15	16	S
17	Spiramycin (SP)	100	16	17-19	20	12	R
18	Penicillin G (P)	10	21	22-28	29	0	R

Table (6): Treatment trails of infected laying hens with *H. alvei.*

Age	Group (1) Non treated				Group (4) Treated with fosbac				Group (5) Treated with Garlycin				Group (6) Treated with Both				Group (7) Non treated			
	E	%	Wt	M	E	%	Wt	M	E	%	Wt	M	E	%	Wt	M	E	%	Wt.	M
286	2	40	45	-	0	0	-		0	0	0	-	0	0	0	-	1	20	30	-
287	3	60	45.66	-	2	40	49	-	0	0	0	-	1	20	44.4	-	1	20	39	-
288	4	80	50	-	1	20	50	-	0	0	0	-	2	40	45	-	1	20	45	-
289	1	20	45	-	1	20	43.2	-	0	0	0	-	5	100	49.5	-	2	40	38.2	-
290	5	100	47.5	-	2	40	44.5	-	0	0	0	-	2	40	48.6	-	2	40	30	-
291	3	60	0	-	2	40	50	-	0	0	0	-	4	80	50	-	0	20	40	-
292	4	80	49	-	4	80	50	-	0	0	0	-	2	40	50	-	1	20	37	-
293	3	60	44.66	-	2	40	47	-	0	0	0	-	4	80	49.6	-	2	40	41	-
294	1	20	50	-	3	60	45	-	3	60	50	-	3	60	50	-	0	0	0	-
295	2	40	50	-	1	20	48.7	-	4	80	49.5	-	5	100	50	-	1	20	42.2	-

E=no. of eggs

%= egg production percentage

Wt= mean egg weight

M= mortalities

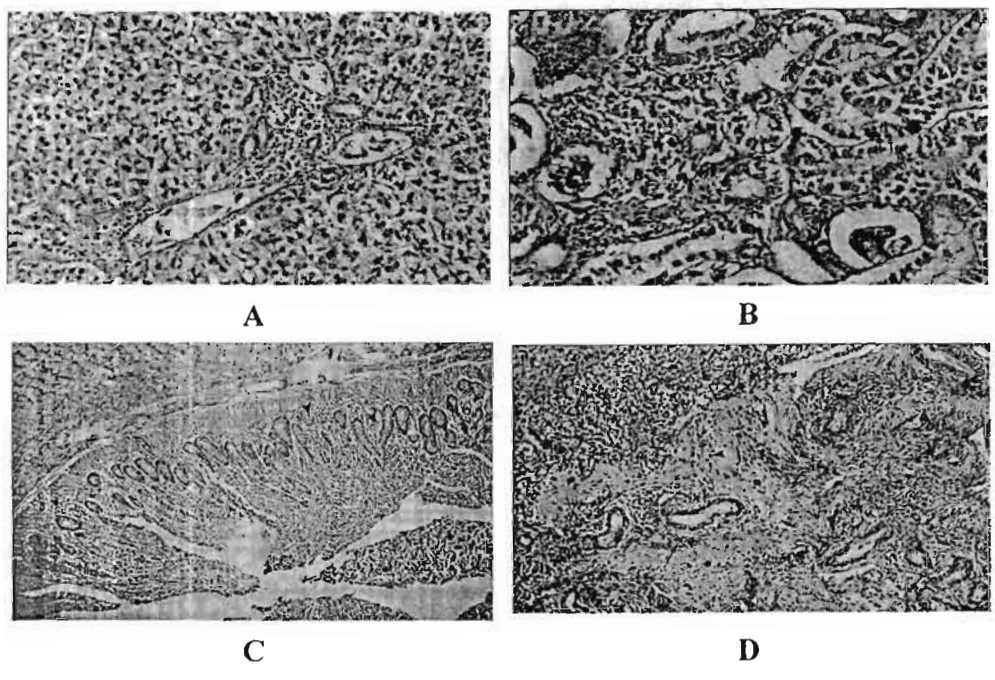


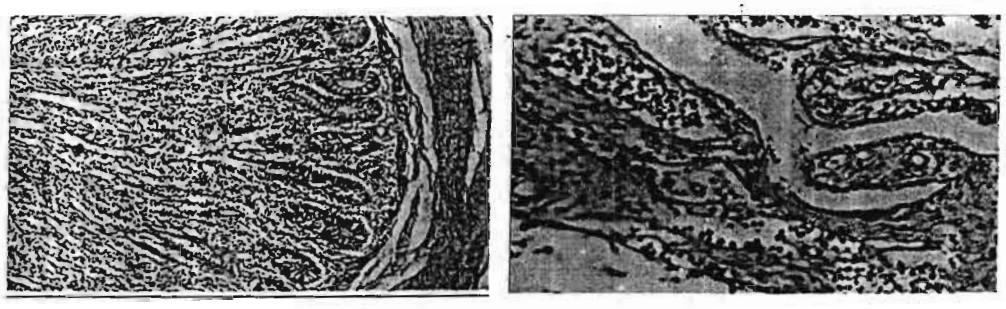
Fig. (2):

(A) Liver of chicken experimentally infected with *Hafnia alvei* on 39 weeks old, showing vacuolar and hydropic degeneration (arrow) of the hepatocytes. Note also pyknosis (arrow) of the nuclei of individual hepatocytes. H&E stain x 400.

(B) Kidney of chicken experimentally infected with *Hafnia alvei* on 39 weeks old, showing periglomerular lymphocytic cellular aggregates (arrow head). Note also coagulative necrosis (arrow) of the lining epithelium of some renal tubules. H&E stain x 400.

(C) Intestine of chicken experimentally infected with *Hafnia alvei* on 39 weeks old, showing desquamation of the lining epithelium (asterisk) and leucocytic cellular infiltration (arrow head) of the lamina propria. H&E stain x 100.

(D) Ovary of chicken experimentally infected with *Hafnia alvei* on 39 weeks old, showing interstitial fibrin deposition and edema (asterisk) admixed with exravasated erythrocytes (arrow) and inflammatory cells mainly macrophages (arrow head). H&E stain x 200.



A B

Fig. (3):

(A) Intestine of infected chicken treated with fosfomycin and tylosin showing leucocytic cellular infiltration (arrow head) of the lamina propria. H&E stain x 200.

(B) Ovary of infected chicken treated with fosfomycin and tylosin showing the interstitial eosinophilic (arrow head) cellular infiltration. H&E stain x 400.

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بعض الدراسات علي عدوي الهافنيا ـ الفي في الدجاج في محافظة الغربية

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استهدفت الدراسة معرفة وبائية المرض وطرق العلاج المناسبة لعدوي الهافنيا الفي في دجاج البياض. لهذا الغرض تم الفحص البكتريولوجي للعينات التي تم تجميعها من 500 دجاجة بياض من مزارع مختلفة في محافظة الغربية هذة المزارع تعاني من هبوط في انتج البيض وانخفاض نسبة الفقس واسهال واعراض عصبية ونفوق .

تم عزل ميكروب الهافنيا الفي بنسبة 3.6٪ من اجمالي المزارع وكان عدد المعزولات ثمانية عشر من خلال الفحص المعملي.

تم عمل اختبار الحساسية ومعرفة اقوى المضادات الحيوية ووجد ان ميكروب الهافنيا الفي حساس لكل من الفوسفومايسن + تيلوزين، النورفلوكساسين، السيفوتاكسيم، السيبروفلوكساسين، الكوليستين،اسبيكتينوميسين والجينتاميسين بينما مقاوم لكل من الكليندامايسين، البينيسيلين، اللينكوميسين، السبيرامايسين،الاموكسيلين،الدوكسيسيكلن،السيفرادين والكانامايسين.

اجريت العدوي الاصطناعية علي 40 دجاجة بلدي بياض عمر 36 اسبوع وملاحظتهم لمدة 50 يوم وتسجيل الاعراض الظاهرية وعمل الصفة التشريحية واعادة عزل الميكروب مرة اخري، ايضا عمل فحص الهستوباثولوجي لعينات من الاعضاء المصابة (الكبد- المبيض- الرئة- القلب- الامعاء- الطحال.....الخ).

اخيرا علاج الدجاج المصاب اصطناعيا باقوى المضادات الحيوية الاكثر تاثيرا علي الميكروب بناءا علي نتيجة اختبار الحساسية، واجري العلاج باستخدام الفوسفوميسين + تيلوزين ومستحضر طبيعي (الجارليسين).