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HAFNIA ALVEI INFECTION IN BROILER BREEDER CHICKENS .

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SUMMARY

The significance of Hafnia alvei (H.alvei) as a new potent poultry pathogen primarly investigated in broiler breeder chickens. H.alvei has been isolated from 3 naturally infected broiler breeder chickens suffering from sudden mortalities , drop in egg production with necrotizing hepatitis and splenitis . Bacteriological examination of cloacal swabs of other apparantly healthy 10 broiler breeder flocks revealed H.alvei with an isolation rate of 8% . Egg transmission of H.alvei could be proved both in naturally and experimentally infected broiler breeders . The antibiogramme of the isolated strains was investigated. Serological screening of the 13 investigated flocks for infections commonly encounted in broiler parent flocks was carried out . Histopathological examination for both naturally and experimentally infected chickens were studied . The pathogenicity of the isolated H.alvei was investigated .

INTRODUCTION

H.alvei is a widespread Gram-negative bacterium, classified as a causative agent of intestinal disorders, which is found in mammals (Binde and Hermansen, 1982 ; Ximena and Oriele , 1983; Mukherjee et al., 1986 ; Sharma et al., 1991; Real et al., 1997) , fish (Sanders and Fryer, 1988), natural environments such as soil, sewage and water (Allen, 1982; Allen et al., 1983 ; Sakazaki and Tamura, 1992) . This organism has been isolated from different foods, including cow's milk (Texdorf et al., 1975) , honey (Salimov, 1978), corned beef (Refaie et al., 1993) and hard goat cheese (Tornadijo et al., 1993), where it affects the microbiologic quality of these food items . Moreover, this microoganism has been reported to cause haemorrhagic septicemia in rainbow trout (Gelev et al., 1990) and has been isolated from aborted equine fetuses (Mukherjee et al., 1986 ; Ximena and Oriele, 1983) as well as from goats suffering

from caprine pneumonia (Sharma et al., 1991) and cows with mastitis (Binde and Hermansen, 1982) . In humans , Hafnia alvei has been associated with gastroenteritis , septicemia, and urinary infections (Krieg and Sneath, 1994; Sakazaki and Tamura, 1992) .

On the other hand, till the year, 1996, H.alvei has not been reported to be associated with any clinicopathologic effects in poultry, however one report arose from spain in 1997 dealing with a case of septicemia associated with H.alvei in laying hens (Real et al.,1997) .

It seems that the role of this organism as a primary pathogen has been questioned in most reported cases; it has generally been considered to be an opportunistic pathogen occurring with other underlying illnesses or predisposing factors (Sakazaki and Tamura, 1992) .

In addition , the septicemic lesions caused by Hafnia spp. in several animal species are very similar to those produced by salmonella spp. (Kelly, 1993; Ridell, 1987). The aim of the present study is to explore such new problem facing poultry industry through reporting the isolation and identification of H.alvei from the internal organs of 3 meat-type breeder flocks suspected clinicopathologically to suffer from this disease . Trials for isolation were also carried out from cloacal swabs from other 10 meat-type breeder flocks . Also, results of gross pathology , histopathological and serological investigations are reported . In addition, the possibility of vertical transmission of H.alvei naturally and experimentally was investigated .

MATERIALS AND METHODS

History of examined chicken farms:

A total of 13 broiler parent chicken flocks located in 6 governorates in Egypt naturally suffering from drops in egg production, decreased hatachability, inappetence, opisthotonus and mortalities were investigated during the peroid of 1999 - 2001. Further details on the history of these flocks are given elsewhere (Table 1) .

Postmortem examination and specimens collection :

(1) Postmortem examination was performed on variable number of freshly dead and moribund birds from 3 suspected flocks (No. 1-3). Gross lesions were recorded and specimens from liver, spleen, kidney, small intestine , cecum and ovary , were collected for isolation .

(2) Cloacal swabs were collected from 10 other flocks (No.4 to 13) and were used for isolation .

(3) Unincubated eggs , non - fertile and fertile eggs, dead embryos and day-old-chicks were collected from the hatcheries of 3 H.alvei naturally infected flocks (No.1,2,3) for isolation

trials to study the possibility of egg transmission .

(4) Fifteen blood samples per flock were collected for serological screening for other related infections .

Histopathological Studies :

Samples from liver, spleen, lungs, kidney, intestines, ovary, heart and brain 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 5-6 micrometer were then cut and stained with haematoxylin and eosin (H&E) stain according to Lillie (1984).

Bacteriological Examination :

Samples were cultured on brain heart infusion broth , selenite F broth, pasteurella broth, peptone water , MacConkeyís agar, Salmonella-Shigella (S.S.) agar , 10% sheep blood agar , nutrient agar , urea agar and triple sugar iron agar. All cultured media were incubated at 37 0C for 48 hours . The isolated bacteria were identified by culture morphology , Gram-stain and Biochemically according to Smibert and krieg (1981) . Biochemical microtest system (API 20 NE Strip ; Bio Merieux, Lyon, France) were also used . To ensure the identification of the isolated bacteria as H.alvei, a Hafnia-specific bacteriophage test using phage 1672(ATCC) was applied according to Guinfe and Valkenburg (1968) . This test allows a rapid distinguishing of Hafnia strains from other similar Enterobacteriaceae specially salmonella and this phage should only show specific plaques of lysis with Hafnia strain which provides a reliable tool for the identification of Hafnia strains .

Serological screening :

This was used for monitoring of other related infections commonly encounted in broiler parent chickens . Serum samples from the investigated farms were screened by ELISA for antibodies to newcastle disease virus (NDV), infectious bronchitis virus (IBV), mycoplasma galisepticum (MG), pasteurella multocida (PM) , and salmonella enteritidis (S.ent.), using commercial ELISA Kits supplied by IDEXX laboratories, Inc., Westbrook, ME04092 and for antibodies to EDS-76 , using commercial ELISA Kits supplied by Bio-Chek laboratories, Oostharen 17B, NL-2801 PC Gouda, Holland . Application and interpretation of the tests were according to the instructions of the Kits producers .

Antibiogramme :

The antibiogramme of 76 H.alvei isolates was investigated against 16 antimicrobial agents using the disc diffusion technique according to Cruickshank et al. (1975) . The test procedure was that recommended by the National Committee for Clinical Laboratory Standards (1990) .

Pathogenicity test :

Thirty one female and 4 male, 31-week-old broiler

breeder chickens were floor reared and used for pathogenicity testing . One female and one male out of these birds were randomly sacrificed and subjected to bacteriological examination to prove their freedom from H.alvei and other pathogens . At 32 weeks of age , the other 33 birds were divided into 3 equal groups consisting of 10 female and 1 male each . Chickens of group 1 were inoculated orally with H.alvei in a dose of 3x108 organisms (Real et al., 1997) . Those of group 2 were inoculated intraperitoneally with a dose of 3x108 H.alvei (Real et al., 1997) , while those of the last group (3) were kept without infection as a control ; three female were inoculated orally and three were inoculated intraperitoneally with 1 ml of sterile saline solution each . Birds of all groups were kept for 4 weeks observation peroid during which the produced fertile eggs were incubated and hatched ; clinical signs, mortality and egg production were recorded . Dead and sacrificed birds at the end of observation peroid as well as unincubated eggs , non-fertile and fertile eggs, dead embryos and day-old chicks were subjected to postmortem, bacteriological and histopathological examination . lesion scores was calculated as fallows :

Liver : 0 = No abnormality .
1 = Enlarged .
2 = Enlarged with randomly scattered whitish-yellow necrotic foci, throughout the parenchyma .

Spleen : 0 = No abnormality .
1 = Slight enlargment .
2 = Enlarged twice as the normal size .

Intestine : 0 = No abnormality .
1 = A diffuse thickening of the intestinal wall .
2 = A diffuse thickening of the intestinal wall with catarrhal exudate on the mucosal surface .

Ovary : 0 = No abnormality.
1 = Oophoritis .
2 = Oophoritis , salpingitis and egg peritonitis .

RESULTS

(A) Epidemiological features of Hafnia alvei infected farms :

During the peroid 1999-2001, 13 H.alvei suspected farms were investigated . They were located in 6 governorates and involved 13 adult broiler breeder farms of 5 locally produced breeds as shown in tables (1 and 5). The examined birds suffered from loss of appetite , diarrhoea , opisthotonus and sudden mortality of 2.9-6.7% per one week prior to the investigation . In addition, all farms showed lower egg production levels which characterized by it's capricious in nature (8.2-19.4%) and 5-9% lower hatchability with no changes in egg shells .

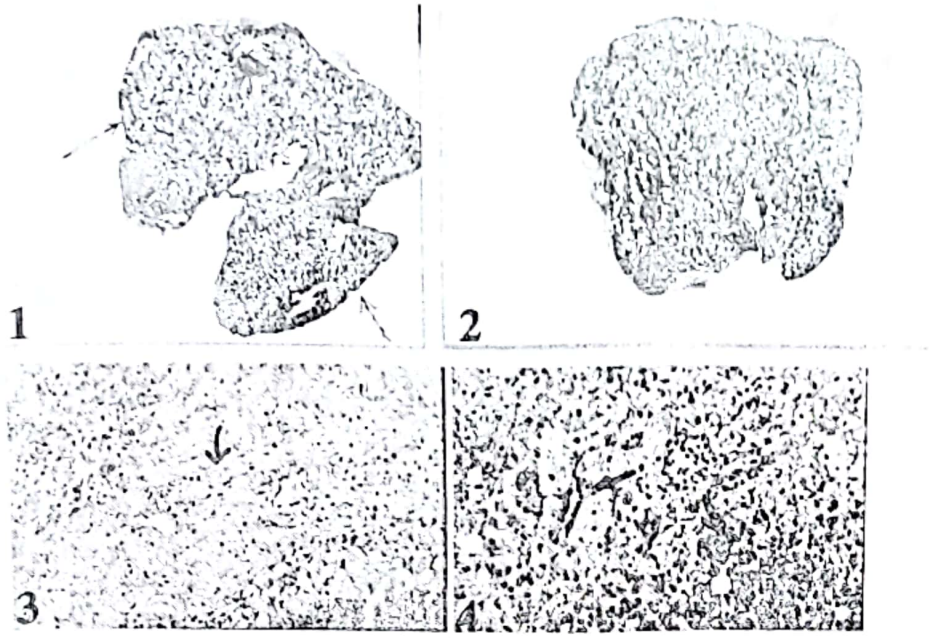

Fig. (1) : Liver of 52-wk-old broiler breeder chicken naturally infected with Hafnia alvei, showing enlargement with randomly scattered whitish-yellow foci, 4-5 mm in diameter throughout the parenchyma .

Fig. (2) : Liver of 41-wk-old broiler breeder chicken naturally infected with Hafnia alvei, showing enlargement with numerous randomly scattered whitish-yellow foci, 4-5 mm in diameter throughout the parenchyma .

Fig. (3) : Section in liver of broiler breeder chicken naturally infected with H. alvei, showing severe multifocal coagulative necrosis, with abundant cell debris and heterophilic infiltration (H&E x 250)

Fig. (4) : Section in liver of broiler breeder chicken naturally infected with H. alvei, showing several multinucleated giant cells (H&E x 400) .

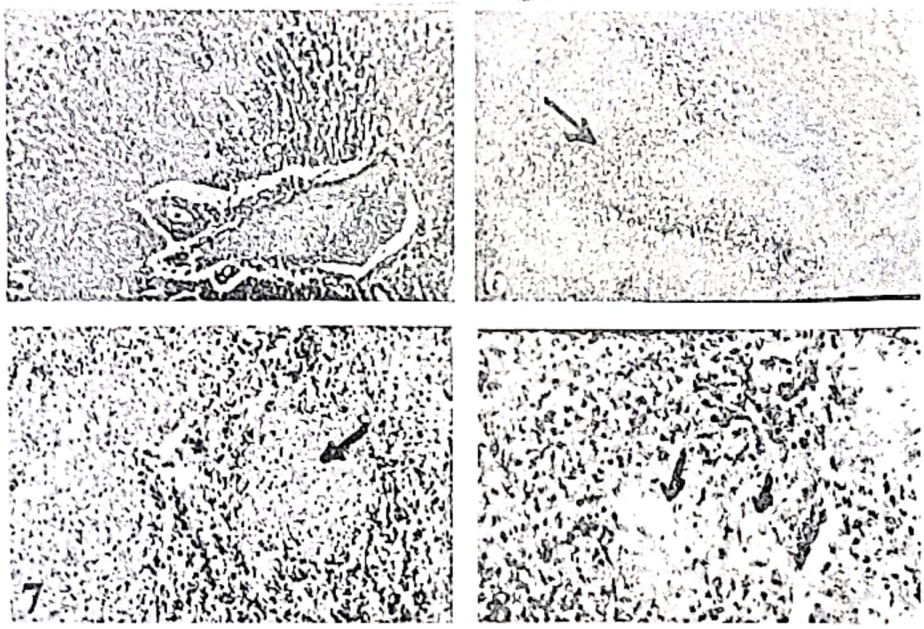

Fig. (5) : Section in liver of broiler breeder chicken naturally infected with H. alvei, showing hyperplasia of bile ductules and disreption of centrilobular hepatocellular cords . (H&E x 100) .

Fig. (6) : Section in liver of broiler breeder chicken naturally infected with H. alvei, showing granuloma (H&E x 100) .

Fig. (7) : Section in spleen of broiler breeder chicken naturally infected with H. alvei, showing severe multifocal necrosis. (H&E x 250) .

Fig. (8) : Section in spleen of broiler breeder chicken naturally infected with H. alvei, showing amyloidosis (H&E x 400) .

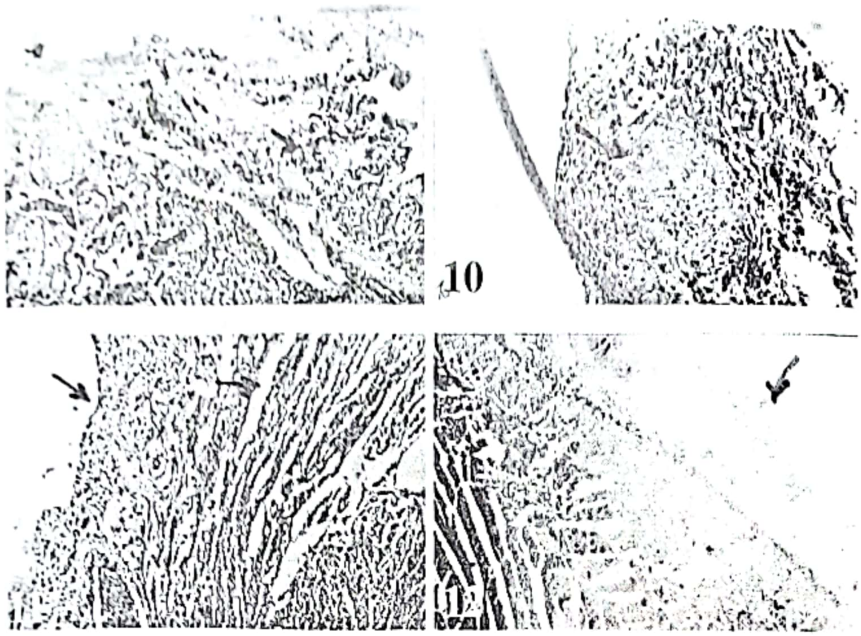

Fig. (9) : Section in lung of of broiler breeder chicken naturally infected with H. alvei, showing emphysema, inflammatory cells and connective tissue proliferation (H&E x 100) .
Fig. (10) : Section in lung of broiler breeder chicken naturally infected with H. alvei, showing granuloma and heterophilic infiltration (H&E x 100) .
Fig. (11) : Section in heart of broiler breeder chicken naturally infected with H. alvei, showing pericarditis and oedema. (H&E x 100) .
Fig. (12) : Section in heart of broiler breeder chicken naturally infected with H. alvei, showing severe pericarditis . (H&E x 40) .

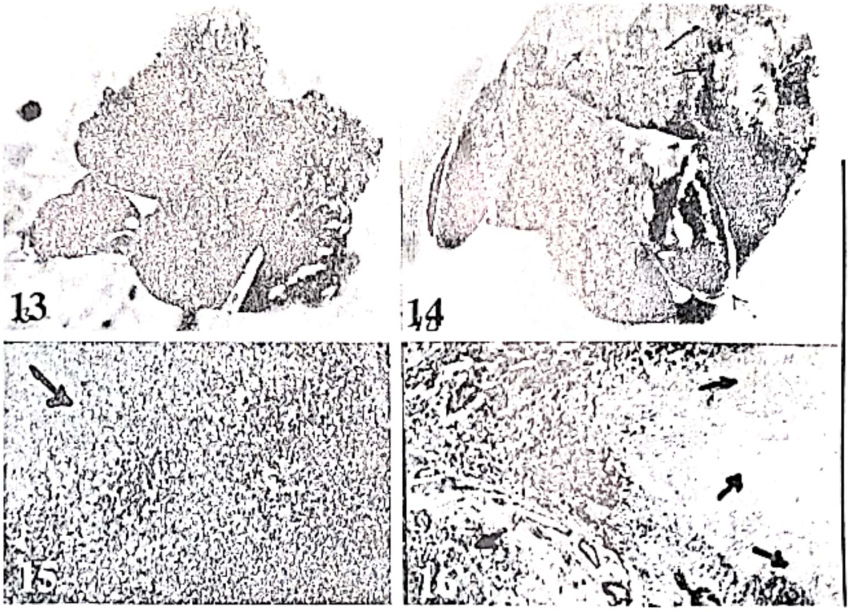

Fig. (13) : Liver and spleen of 36-wk-old broiler breeder chicken experimentally infected with H. alvei, showing enlargement of liver and spleen with randomly scattered whitish ñ yellow foci, 4-5 mm in diameter throughout the parenchyma .
Fig. (14) : Liver and gall blader of 36-wk-old broiler breeder chicken experimentally infected with H. alvei, showing enlargement of liver as well as distension of gall bloder with randomly scattered whitish ñ yellow foci, 4-5 mm in diameter throughout the liver parenchyma .
Fig. (15) : Section in liver of broiler breeder chicken experimentally infected with H. alvei, showing nultifocal coagulative necrosis with heterophilic infiltration (H&Ex100) .
Fig. (16) : Section in liver of broiler breeder chicken experimentally infected with H. alvei, showing several granulomas with fibroblastic proliferation . (H&Ex100) .

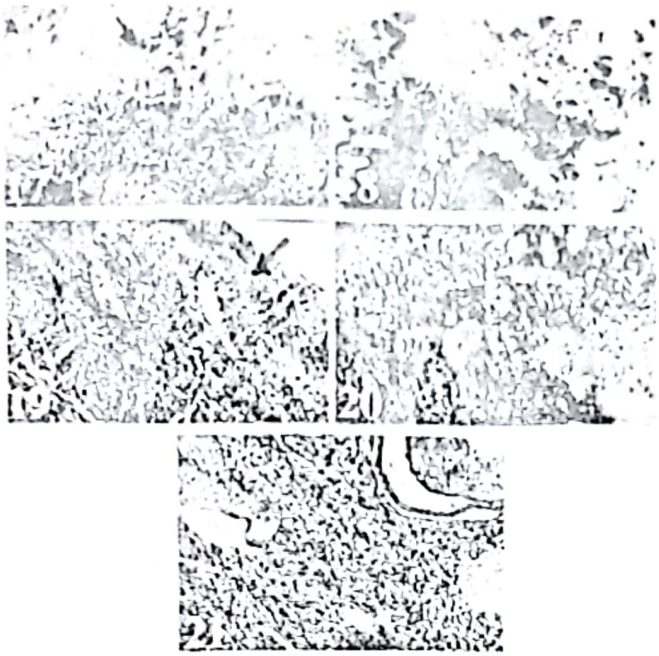

Fig. (17) : Section in spleen of broiler breeder chicken experimentally infected with H. alvei, showing haemorrhages and nultifocal necrosis . (H&Ex250) .
Fig. (18) : Section in lung of broiler breeder chicken experimentally infected with H. alvei, showing congestion, emphysema and haemorrhages with heterophilic infiltration . (H&Ex100) .
Fig. (19) : Section in heart of broiler breeder chicken experimentally infected with H. alvei, showing severe pericarditis . (H&Ex100) .
Fig. (20) : Section in brain of broiler breeder chicken experimentally infected with H. alvei, showing demylination and neural degeneration . (H&Ex100) .
Fig. (21) : Section in vary of broiler breeder chicken experimentally infected with H. alvei, showing proliferation of fibroblast . (H&Ex100) .

(B) Gross Lesions :

Postmortem examination of dead and sacrificed birds revealed very characteristic lesions in the liver, which was enlarged with numerous randomly scattered whitish-yellow foci, 4-5 mm in diameter, throughout the parenchyma (Fig.1-2). Oophoritis, pericarditis and pneumonia were also recorded . Splenomegaly (twice the normal size) and a diffuse thickening of the intestinal wall with catarrhal exudate on the mucosal surface were also observed .

(C) Histopathological examination :

(1) Liver : Showing severe multifocal coagulative necrosis , blood vessels engorged with blood and contain heterophils and oesinophils, pronounced granulomas appeared in the parenchyma containing inflammatory cells and giant cells and activation of the kupher cells . Perivascular granulocytes and lymphocytes infiltration together with histeocytes , and pronounced hyperplasia of bile ductules with connective tissue proliferation (Fig.3-6) .

(2) Spleen : Showing focal necrosis, amyloidosis, haemorrhage, thickening of the splenic capsule with subcapsular oedema and mild depletion of lymphocytes (Fig.7-8).

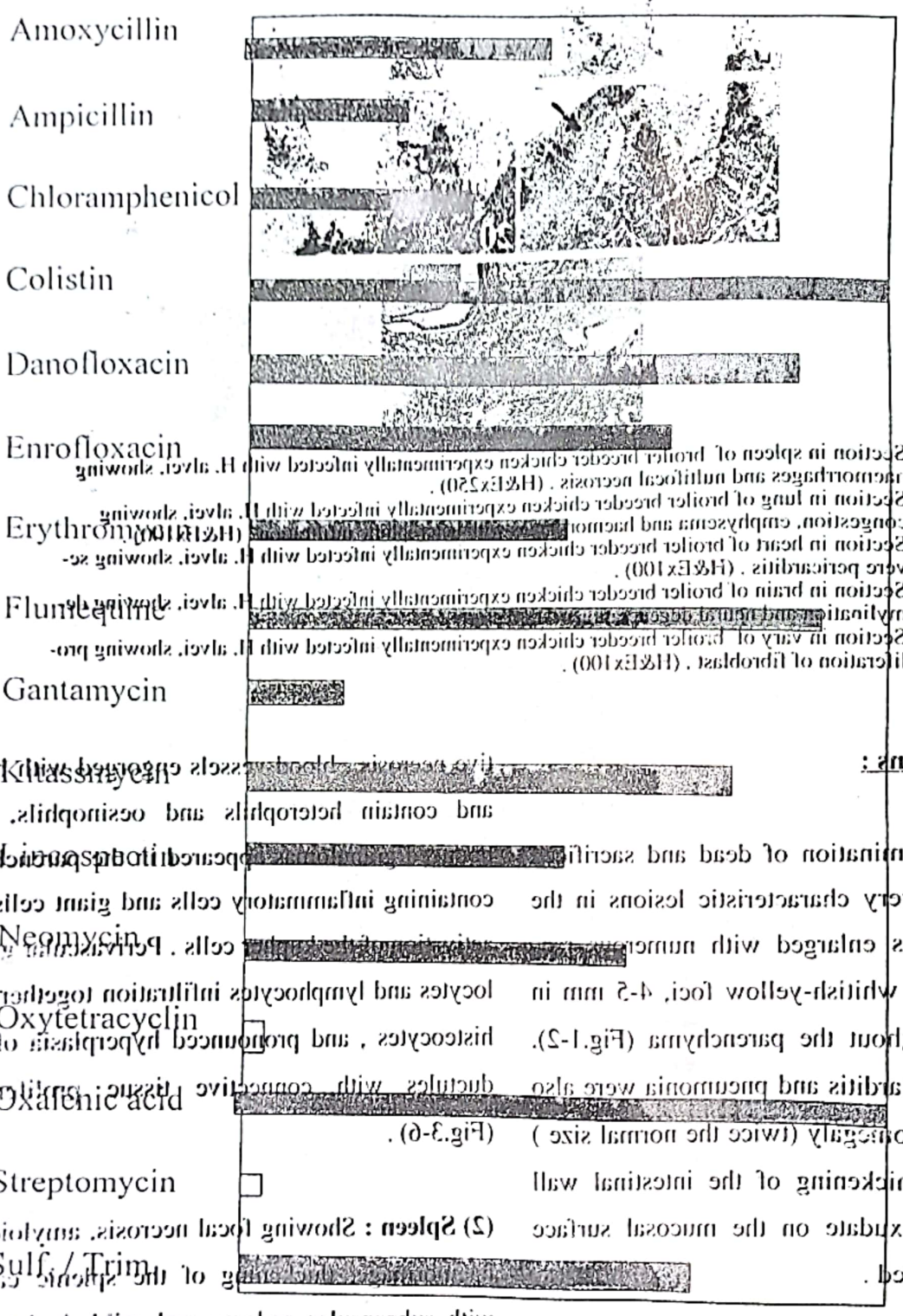

Fig. (22): Results of in-vitro sensitivity of H.alvei isolates (n=76) to different antimicrobial agents

(3) Lung : Showing severe haemorrhage and emphysema . The lung tissues infiltrated with inflammatory cells mainly heterophils and plasma cells, and connective tissue proliferation (Fig.9-10) .

(4) Heart : Showing severe pericarditis . The pericardium and myocardium were infiltrated with granulocytes and lymphocytes (Fig.11-12) .

(5) Ovary : Showing diminished yolk sacs and fibroblastic proliferation .

(6) Intestine : Showing hyperemia and diffuse catarrhal enteritis with loss of epithelial cells and heterophils infiltrating the lamina propria .

(7) Kidneys: Showing intertubular congestion with inflammatory cells infiltration especially lymphocytes . Denudation of the tubular eptheli-um and clowdy swelling were seen .

(8) Brain : Showing congestion of the blood vessels, gliosis , demylination and neural degeneration .

Table (1): History of examined broiler parents of investigated chicken flocks.

Code No.	Locality	Bird** variety	House capacity No.	Age/ Weeks	Mortality %* per week	Drop in egg Prod. % per week	Hatchability % wk	Vaccination Schedule
1	Sharkia	A	30.000	52	6.7	19.4	67	IB-ND-EDS.
2	Sharkia	B	30.000	41	5.9	15.8	75	IB-ND-EDS.
3	Sharkia	B	20.000	38	5.1	16.3	76	IB-ND.
4	Sharkia	A	20.000	49	4.8	8.2	71	IB-ND-EDS.
5	Monofia	B	10.000	45	5.3	12.5	70	IB-ND.
6	Gharbia	B	12.000	57	3.8	12.3	43	IB-ND.
7	Gharbia	C	12.000	32	4.2	16.7	71	IB-ND-EDS.
8	Gharbia	D	12.000	43	4.4	11.9	68	IB-ND
9	Gharbia	E	12.000	49	3.5	13.6	69	IB-ND-EDS.
10	Ismalia	B	25.000	39	5.6	14.3	73	IB-ND
11	Giza	C	20.000	44	3.3	14.7	64	IB-ND-EDS.
12	Alexandria	A	20.000	53	2.9	11.0	47	IB-ND
13	Alexandria	C	20.000	36	4.7	14.3	74	IB-ND-EDS.

* Mortality: Recorded percentage during one week prior to the investigation.
** A-E= Represent different breeds.
Drop in egg production: Recorded percentage as compared to a week before.
Vaccination schedule : The flocks were vaccinated by combined inactivted vaccine against ND, IB and EDS at point of lay, and were monthly vaccinated against ND with LaSotea live vaccine and every 3 months with MA5 live IB vaccine as well as application the schedule of vaccination according to the company perducer as Marek's, Pox, ILT, Gumboro, AE, Fowl cholero, Infectious coryza and Reo vaccines.

Table (2): Serological monitoring of investigated broiler parent chicken flocks using Blocking ELISA assay.

Code No.	Age/ Weeks	Sera# No.	NDV		IBV		PM *		EDS-S76			MG ^		S. ent.	
			GM*	CV** %	GM	VC%	GM	VC%	GM	VC%	+ve No.@/ Exam. No.	+ve No./ Exam. No.	%	+ve No Exam. No.	%
1	52	15	11.173	17.8	5.382	30.0	1.467	47.0	2.891	33.0	14/14	1/14	7.1	0/14	0.0
2	41	15	9.855	21.2	4.991	27.2	1.727	41.3	3.121	28.7	15/15	0/15	0.0	0/15	0.0
3	38	15	10.433	19.5	4.656	20.7	2.311	37.8	0.349	96.6	3/15	4/15	26.7	0/15	0.0
4	49	15	10.256	20.7	5.853	19.9	1.939	43.7	2.772	31.3	13/13	3/13	23.1	0/13	0.0
5	45	15	8.937	32.4	4.358	23.8	2.721	38.9	0.297	88.9	0/15	6/15	40.0	0/15	0.0
6	57	15	11.255	24.6	5.437	28.3	2.121	48.4	0.392	92.5	4/14	5/14	35.7	0/14	0.0
7	32	15	10.944	36.3	4.765	29.4	1.665	41.9	3.462	30.6	15/15	1/15	6.7	0/15	0.0
8	43	15	9.249	14.9	4.859	33.8	1.874	39.2	0.249	95.3	0/15	5/15	33.3	0/15	0.0
9	49	15	9.667	18.4	5.271	22.4	1.965	40.7	3.227	29.8	15/15	7/15	46.7	0/15	0.0
10	39	15	11.335	27.8	5.346	27.6	2.834	36.6	0.368	89.9	0/15	2/15	13.3	0/15	0.0
11	44	15	8.446	35.4	4.943	30.3	2.747	39.5	2.848	27.5	14/14	5/14	35.7	0/14	0.0
12	53	15	10.567	23.5	4.667	29.8	2.431	41.4	0.572	94.6	6/15	8/15	53.3	0/15	0.0
13	36	15	10.735	26.6	5.195	34.1	2.913	38.8	3.253	25.2	15/15	1/15	6.7	0/15	0.0

* GM = Geometeric mean ** CV= Coefficient variation. @ Positive number/Examined number.
\# No. = Number.
NDV = NewCastle disease virus.
IBV = Infectious bronchitis virus.
* PM = Pasteurella multocida.
EDS = 76 = Egg drop syndrome - 76.
^ MG = Mycoplasma gallisepticum.
S.ent. = Salmonella enteritidis.

Table (3): Incidence of H.alvei isolation from various organs of naturally infected broiler breeder flocks.

Flock No.	Bird Exam. No.	Cultures from organs						Total H.alvei isolation	Rate of H.alvei isolation
		Liver	Spleen	Kidney	Small intestine	Cecum	Ovary		
1	9	4/6*	2/7	1/8	2/5	1/4	2/5	12/35	34.3
2	10	3/7	2/6	2/9	3/6	2/6	1/7	13/41	3.07
3	10	4/7	3/8	1/7	2/5	1/5	3/6	14/38	36/8
Total		11/20	7/21	4/24	7/16	4/15	6/18	39/114	
Percentage		55.00	33.3	16.7	43.8	26.7	33.3	34.2	34.2

* Positive number/Examined number.
No. = Number.

Table (4): Incidence of H.alvei isolation from cloacal swab cultures from apparantlly healthy broiler breeder flocks.

Flock No.	Bird Exam. No.	Cloacal swab cultures ◊
4	10	0/10
5	10	2/10
6	10	0/10
7	10	3/10
8	10	0/10
9	10	2/10
10	10	0/10
11	10	0/10
12	10	0/10
13	10	1/10
Total 10	100	8
Percentage		8

◊ Positive number/Examined number.
No. = Number

Table (5): Incidence of H.alvei isolation from eggs and hatched chicks produced by naturally infected broiler parent flocks.

Flock No.	Eggs			Hatched chicks					Total
	Unincubated	Non-fertile	Dead embryos	Liver	Heart blood	Small intestine	Cecum	Gall blader	Yolk sac	
1	2/10*	1/10	3/10	3/10	3/10	1/10	0/10	1/10	1/10	15/90
2	1/10	1/10	1/10	1/10	1/10	0/10	0/10	1/10	1/10	7/90
3	1/10	1/10	1/10	2/10	2/10	0/10	0/10	0/10	0/10	7/90
Total	4/30	3.30	5/30	6/30	6/30	1/30	0/30	2/30	2/30	29/270
Percentage	13.3	10.0	16.7	20.0	20.0	3.3	0.0	6.7	6.7	10.7

* Positive number/Examined number.
No. = Number.

Table (6a): Biochemical characteristics of bacteria isolated from broiler breeder chickens.

Tests		Isolates of greyish convex colonies on macConkey agar plates.																																					
		From various internal organs																																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
API* 20NE	ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+		
	LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
	ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
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	RHA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
	SAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	MEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	AMY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	ARA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
	OX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+								
		Hafnia alvei																																						

* Code number : 07387. Lot-ch-B. 9443125 49.

Table (6b): Cont. Biochemical characteristics of bacteria isolated from broiler breeder chickens.

Tests		Isolates of greyish convex colonies on macConkey agar plates.																												
		From swab cultures								From eggs and hatched chicks																				
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
API* 20NE	ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ADH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	CIT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	H2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	URE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	IND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	VP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	GEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	MAN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	INO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RHA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AMY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	ARA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
The isolated Pathogen	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Hafnia alvei																												

* Code number : 07387. Lot-ch-B. 9443125491.

ONPG = Beta-galactocidase.
ADH = Arginine hydrolase
LDC = Lysine decarboxylase
ODC = Ornithine decarboxylase.
CIT = Citrate utilization.
H2S = H2S Production.
URE = Urease.
TDA = Tryptophane desaminase.
IND = Indole production.
VP = Vogus Proskour.
GEL = Gelatinase.
GLU = Glucose [fermentation / oxidation].
MAN = Manitol [fermentation / oxidation].
INO = Inositol [fermentation / oxidation].
SOR = Sorbitol [fermentation / oxidation].
RHA = Rhamnose [fermentation / oxidation].
SAC = SAC = Sucrose [fermentation/oxidation].
MEL = Melliose [fermentation / oxidation].
AMY= Amygdalin [fermentation/oxidation].
ARA = Arabinose [fermentation / oxidation].
OX = Cytochrome oxidase

Table (7): Experimental infection of broiler breeder chickens with Hafnia alvei (H. alvei).

Group No.	Lesion score 4 weeks PI			Mortalities Per weeks				Total		Total egg Production / week %								Reisolation of	Reisolation from	
	L	S	I	O	1st wk	2nd wk	3rd wk	4th wk	No.	%	1st week		2nd week		3rd week		4th week			
											% of egg prod.	% of drop in egg prod.	% of egg prod.	% of drop in egg prod.	% of egg prod.	% of drop in egg prod.	% of egg prod.	% of drop in egg prod.	No.	%	No.	%
1	3	1	2	1	1	-	-	-	1	9.1	8 eggs = 80%	10 %	8 eggs = 80%	10 %	8 eggs = 80%	10 %	7 eggs = 70%	10 %	7/11	63.6	62/81	52.1%
2	3	2	1	1	1	1	-	-	2	18.2	8 eggs = 80%	10%	7 eggs = 70%	20%	6 eggs = 70%	20 %	6 eggs = 80%	20 %				
3	0	0	0	0	-	-	-	-	0	0.0	9 eggs = 90%	0 %	9 eggs 90%	0%	8 eggs 80%	0 %	8eggs = 80%	0 %				

L = Liver. S = Spleen. I = Intestine. O = Ovary. PI = Post infection.

* As compared to control birds.

(D) Serological results :

The results of serological screening for other related infections are shown in table (2) .

(E) Bacteriological examination :

Hafnia alvei isolation from various internal organs of three suspected broiler parent flocks was successful (Table 2). Thirty-nine isolates, which grow on macConkey and S.S. agar and revealed motile Gram-negative short rods, could be recoverd from liver,spleen, Kidney, small intestine, cecum and ovary . Colonies appeared as grey, convex and were 1-2 mm in diameter . These colonies were able to grow well on ordinary media . The frequency of isolation from the various organs is shown in table(3). Results of H.alvei isolation from cloacal swab cultures from other 10 broiler breeder chicken flocks revealed that 4 flocks were positive and yielded 8 isolates (Table 4) .

Trials for isolation of H.alvei from unincubated eggs, non-fertile eggs, dead embryos and hatched chicks from 3 out of the 13 broiler breeder chicken flocks were successful and revealed 29 isolates (Table 5) .

The biochemical characteristics of the isolated organisms are shown in table (6) .

Hafnia phage on nutrient agar did not lyse any bacteria other than Hafnia at a dose 1x10-6 and produce four clear plaques of lysis with a zone of 1-2 mm in diameter which confirm the organism .

(F) Antibiogramme results :

Results of sensitivity testing of 76 H.alvei isolates are shown in Fig.(22). All tested isolates (100%) showed high sensitivity to colistin and oxolonic acid while 80% of them were sensitive to danofloxacin and flumequine, 70% to kitassmycin and sulfamethoxole plus trimethoprim, 60% to enrofloxacin and neomycin, 50% to erythromycin and lincospectin, 40% to amoxycillin, 30% to chloramphenicol, 20% to ampicillin and 15% to gentamycin. None of the isolates was susceptible to oxytetracyclin and streptomycin .

(G) Experimental results :

Chickens exposed to the experimental infection showed mortality, drop in egg production, opisthotonus and diarrhoea for 7 days after inoculation compared with the control group. Gross lesions were similar to those described in naturally infected chickens (Fig.13-14) . Bacterial reisolation from infected chickens and from eggs and hatched chicks produced by infected chickens were recorded (Table 7) .

Histopathological results were similar to those observed in naturally infected chickens (Fig. 15-21). Birds of the control group remained clinically healthy and showed neither pathological lesions nor H.alvei isolation .

DISCUSSION

Bacterial infections continue to plague the poultry industry and to contribute to human food-borne disease all-over the world despite the world-wide implementation of eradication, biosecurity, vaccination, sanitation, medication programmes and public education (Barrow, 1997).

Hafnia alvei has only recently been recogrized as a pathogen in poultry (Real et al., 1997) . It is Gram-negative short rods and grow on ordinary media as well as some of highly selective media as S.S. agar . The organism was closely resemble to many species of Enterobacteriaceae of clinical interest especially salmonella species through it's morphological, colonial, biochemical characters (Sakazaki and Tamura, 1992) and the septicemic lesions (Kelly, 1993 ; Ridell, 1987). The bacterium could be distinguished from other similar pathogens (Sakazaki and Tamura , 1992; Krieg and sneath, 1994) . Because the organism can cause confusion with salmonella species, the API20 NE microtest system was a useful tool as a first diagnostic step and the Hafnia-specific bacteriophages provided the final reliable and precise method for the identification and differentiation of H.alvei from other bacteria that may cause similar clinicopathologic effects (Real et al., 1997; Rodriguez et al., 1998) .

In the present investigations, H.alvei could be isolated from the internal organs of 3 broiler breeder chicken flocks , which were mainly suffering from loss of appetite, diarrhoea , opisthotonus, capricious drop in egg production and sudden mortality . Similar findings but in laying hens were made by Real et al. (1997). Moreover, we succeeded in the isolation of H.alvei from 4 flocks out of 10 through cloacol swabs culturing, which revealed 8 isolates . Cultures of cloacol swabs for H.alvei proved to be very useful in understanding many aspects of H.alvei infection . The gross pathological lesions in all examined farms revealed that the most characteristic lesions were in the liver which was enlarged and scattered with numerous randamly whitish yellow necrotic foci , 4-5 mm in diameter, throughout the parenchyma as well as splenomegaly twice normal size with scattered necrotic foci. Oophoritis, pericarditis and pneumonia were also observed . Histopathologically, the internal organs were affected as liver showing severe multifocol coagulative necrosis , pronounced granulomas appears in the parenchyma contaiming inflammatory and giant cells with activation of the kupher cells. Other visceral organs revealed focal necrosis, thickening of splenic capsule and mild depletion of lymphocytes in spleen ; severe haemorrhage, emphysema and infiltration with heterophils and plasma cells in lung; percarditis and infilration of pericardium and myocardium with granulocytes and lymphocytes ; fibroblastic proliferation in ovary ; hyperemia, diffuse catarrhal enteritis with loss of epithelial cells and heterophils infiltrating the lamina propria in intestine ; and intertubular congestion with lymphocytes in-

filtration and denudation of the tubular epithelium with clowdy swelling in kidneys . Another interesting finding in the brain was congestion of the blood vessels, gliosis , demylination and neural degeneration .

These pathological changes strongly confirmed the H.alvei infection and coincided with the findings of Real et al. (1997) .

Our work in these infection arose-out of initial studies on the related infection to explore and clarify the precise role of H.alvei as a primary or secondary or opportunistic pathogen . The serological findings revealed that the all investigated farms were in a good immune status against NDV, IBV and Fowl chalera, and proved to be exposed to MG infection except farm No. 2 . The most remarkable serological findings were detection of serum antibodies against EDS-76 in farm No. 3 , 6 and 12 with no history of previous vaccination .

The significance of these serological findings explain the aggravation of the infection with other pathogen and argue that such infection caused by H.alvei as a primary pathogen , However, no history of predisposing factors or no other pathogen (bacteria) could be isolated from the diseased birds. From the epidemiological stand point of view; unincubated hatching eggs, dead in shell embryos and hatched chicks from 3 naturally infected flocks were positive for isolation of H.alvei . In addition, reisolation of H.alvei from eggs and hatched chicks produced by experimentally infected broiler breeder chickens confirmed that egg transmission could be a way of infection, and probably explains the wide spread of the organism in nature .

This work has recently been extrapolated to H.alvei septicemia in chickens and the epidemiology of the disease still remains a mystery . Regarding the results of H. alvei sensitivity in vitro ; all tested isolates showed high sensitivity to colistin and oxolonic acid . This narrow finding of high antibiotic sensitivity to the organism might be due to increased resistance patterns and residue problems . These reasons concur with Te Winkel (1997) . Furthermore, All isolates were resistant to streptomycin and oxytetracyclin which is in accordance with the finding of Gross and Holmes (1990) in studying sensitivity testing of Enterococci .

On the other hand , experimental infection of H.alvei in susceptible 32-week-old broiler breeder chickens revealed intersting findings in all response variables measured in this trial when compared with the controls . Pathogenicity testing indicated that the H.alvei isolates was capable of inducing mortalities (10% by oral route and 20% by intraperitoneal route) , drop in egg production and diarrhoea which similar to those of naturally infected flocks . Reisolation of H.alvei post experimental exposure to the organism was successful.

Similar observations were made by Real et al. (1997) . Since the isolates were selected from field strains with differing clinical pictures , one may argue that concomitant stressors or infections could exacerbate H.alvei symptoms.

In conclusion, these results are very exciting and open up opportunities for the disease diagnosis, prevention and control for the future . H.alvei is claimed to be a new pathogen isolated for the first time from meat-type breeder chicken flocks . The attitude of the poultry diagnostic people may be ignore or confuse with this organism, so, we try to put a light on such problem . Notwithstanding, the techniques will have to be further developed , in part to automate them for busy diagnostic laboratories , and partly because of the variety exhibited by the pathogens .

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