TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(2): 101-110
© TÜBİTAK
doi:10.3906/vet-0707-1

Pathological and clinical findings and tissue distribution of *Salmonella* Gallinarum infection in turkey poults*

Latife BEYAZ[1,**], Ayhan ATASEVER[1], Fuat AYDIN[2], K. Semih GÜMÜŞSOY[2], Seçil ABAY[2]

[1]Department of Pathology, Faculty of Veterinary Medicine, Erciyes University, 38090 Kayseri - TURKEY

[2]Department of Microbiology, Faculty of Veterinary Medicine, Erciyes University, 38090 Kayseri - TURKEY

Received: 03.07.2007

Abstract: This study aimed to experimentally induce *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* Gallinarum) infection in turkey poults in order to detect *S.* Gallinarum using immunohistochemical, bacteriological, and histological procedures. The study included 90 white turkey poults that were divided into 3 groups (1-day-old, 3-week-old, and 2-month-old), each of which was orally inoculated with an inoculum of 0.3 mL of *S.* Gallinarum strain 9 broth culture that contained approximately 2×10^4, 1×10^6, and 1×10^9 cfu/mL, respectively. The duration of the experiment was 18 days. Clinical, pathological, and bacteriological findings were evaluated daily until 18 days post inoculation (pi). The most evident clinical symptoms in all 3 groups were diarrhea and somnolence, especially in 1-day-old group. *S.* Gallinarum strain 9 was isolated from organs and cloacal swabs up to 16 days pi. Inflammation and typical granulomatous nodules in the internal organs were observed in all 3 groups. Strong immunoreactivity was determined in the lungs, bursa of Fabricius, caecum, ileum, and cloaca of all infected poults up to 18 days pi. In conclusion, *S.* Gallinarum infection caused heterophilic granulomas, especially in the duodenum of turkey poults, and immunohistochemical analysis can be considered as an adjunct to bacteriological methods in the diagnosis of *S.* Gallinarum infection.

Key words: Turkey poults, *Salmonella* Gallinarum, pathological findings

Hindilerde *Salmonella* Gallinarum enfeksiyonununun patolojik ve klinik bulguları ile doku dağılımı

Özet: Bu çalışma hindilerde deneysel *Salmonella* Gallinarum enfeksiyonu oluşturmak ve *S.* Gallinarum'u immunohistokimyasal, bakteriyolojik ve histopatolojik yöntemlerle belirlemek amacıyla yapıldı. Çalışmada, 90 adet beyaz hindi 1 günlük, 3 haftalık, 2 aylık olmak üzere 3 gruba ayrıldı ve sırasıyla 2×10^4, 1×10^6 ve 1×10^9 kob/mL *S.* Gallinarum 9 suşu içeren inokulumdan 0,3 mL oral olarak inokule edildi. Deney süresi 18 günle sınırlandırıldı. İnokulasyondan sonraki 18. güne kadar her gün bulgular klinik, patolojik, bakteriyolojik yönden değerlendirildi. Tüm gruplarda gözlenen en belirgin semptomlar, özellikle 1 günlük grupta olmak üzere, ishal ve uyuklamaydı. İnokulasyondan sonraki 16. güne kadar *S.* Gallinarum 9 suşu organlardan ve kloakal swaplardan izole edildi. Enfekte gruplardaki hayvanların tamamının iç organlarında yangı ve tipik granulamatöz odaklar gözlendi. İnokulasyandan sonraki 18. güne kadar tüm enfekte hindilerin akciğer, bursa Fabricius, sekum, ileum ve kloakalarında kuvvetli immunoreaksiyon belirlendi. Sonuç olarak, *S.* Gallinarum enfeksiyonu hindilerin özellikle duodenumunda heterofilik granulomlar oluşturmuştur ve *S.* Gallinarum enfeksiyonun tanısında immunohistokimyasal metodun bakteriyolojik metotları destekleyici bir teknik olabileceği düşünülmüştür.

* This project was supported by Erciyes University Scientific Research Project Unit (Project no: VA-02-50-5).
** E-mail: lbeyaz@erciyes.edu.tr

Introduction

Fowl typhoid is a septicemic infection of domestic birds that is caused by *S.* Gallinarum (1,2). The disease exists worldwide and primarily affects chickens and turkeys, but other birds such as quail, pheasants, ducks, peacocks, and guinea fowl are also susceptible (1,3). Morbidity is high among all age groups of the birds, whereas mortality may range from 10% to 90% (4). Although vaccination and various eradication programs have been implemented to protect poultry species from the disease, complete success has not been achieved (5,6). Therefore, *S.* Gallinarum infection causes significant economic losses to the poultry breeding and food production industries (2,3). The diagnosis of this infection is of considerable importance (7). Immunohistochemical techniques have been used for the diagnosis of infections caused by *Salmonella* species in domestic animals, and the causative agents have been indentified (8-11); however, to the best of our knowledge, this is the first study of the diagnosis of *S.* Gallinarum using the immunohistochemical method in turkeys. This study aimed to evaluate the pathological and bacteriological findings of fowl typhoid in turkey poults and to perform the rapid diagnosis of *S.* Gallinarum strain 9 using the immunoperoxidase method.

Materials and methods

Animals

The study included 90 *Salmonella*-free white turkey poults. The animals were divided into 3 groups: 1-day-old, 3-week-old, and 2-month-old. Each group was further divided into 2 subgroups—a control group that consisted of 10 animals and a treatment group that consisted of 20 animals. The animals in all groups were housed on the floor in wire-separated pens, and a commercial grower diet was provided ad libitum throughout the experiment. Control and treatment groups were reared in separate rooms. This study was approved by the Erciyes University, Faculty of Veterinary Medicine Ethics Committee (Approval date: 15.09.2004, Number: 055).

Preparation of inoculum

S. Gallinarum strain 9 (provided by Dr. Paul Barrow, Institute for Animal Health Compton Laboratory, Compton, Nr. Newbury, Berks, England) was inoculated on nutrient agar from an overnight broth culture, and incubated at 37 °C for 24 h. The colonies grown on nutrient agar was suspended in PBS. The method described by Desmidt et al. (12) was adapted to the preparation of the inoculum. Three different inocula were prepared for experimental infection.

Induction of *S.* Gallinarum infection

The birds in the 1-day-old, 3-week-old, and 2-month-old groups were inoculated orally with an inoculum of 0.3 mL of *S.* Gallinarum strain 9 that contained approximately 2×10^4, 1×10^6, and 1×10^9 cfu/mL, respectively.

Sample collection

Two animals from each treatment group and 1 animal from each control group were randomly selected and euthanized via cervical dislocation between the 1st and 18th post-inoculation (pi) day. Tissue samples were collected from the liver, spleen, lungs, heart, kidneys, bursa of Fabricius, intestines, pancreas, gizzard, cloaca, bone marrow, brain, and cerebellum for histological, immunohistochemical, and bacteriological analysis. Cloacal swabs were also collected for bacteriological examination.

Tissue processing for histopathological examinations

Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned (5-6 μm), and then mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) (13) and examined under a light microscope (Olympus BX-50). Sections were also mounted on adhesive slides for immunoperoxidase staining.

Bacteriological examinations

For bacteriological examination, pre-enrichment and direct isolation methods were used simultaneously. For direct isolation of the agent, tissue samples and cloacal swabs were directly inoculated on Brilliant Green Phenol Red Agar (BGFRL) (Merck, Darmstadt, Germany), as well as on MacConkey agar (Merck, Darmstadt, Germany). The inoculated petri dishes were incubated at 37 °C for 48 h. For the pre-enrichment method, samples were pre-enriched in 2% buffered peptone water (BPW) (Merck, Darmstadt, Germany) by incubating overnight at 37 °C, and BPW

cultures were inoculated on Rappaport Vassiliadis (RV) medium (Merck, Darmstadt, Germany) and incubated at 42 °C for 24 h. Then, the culture was inoculated on Brilliant Green Agar (BGA). After 2 days of incubation at 37 °C, the suspected colonies on BGA were screened via biochemical testing and confirmed serologically (13,14).

Production of primary antiserum against *S.* Gallinarum

The primary antiserum was prepared according to the method described by Beyaz and Kutsal (8). Two adult New Zealand rabbits were inoculated 3 times with 0.25, 0.5, and 1 mL of *S.* Gallinarum strain 9 (approximately 7×10^9 cfu/mL) via an ear vein at 5-day intervals. Blood samples were collected from the rabbits on day 15, after the last injection, and sera were separated and stored at –20 °C. Serial dilutions ($\log_2$) of the primary antiserum—from 1/2 to 1/256—were made to obtain optimal primary antibody titers.

Immunohistochemistry for *S.* Gallinarum

The immunoperoxidase technique was performed according to the method of avidin-biotin-peroxidase complex (ABC) described by Porter and Holt (10). Sections were deparaffinized in xylene and dehydrated with graded alcohol. Following quenching of endogenous peroxidase activity with 1% H_2O_2 in methanol for 15 min, 10% normal goat serum (Shandon, Pittsburg, USA) was placed onto sections to block non-specific binding of immunoglobulins. Sections were labeled with rabbit anti-*S.* Gallinarum at a dilution of 1:128 in PBS (pH 7.4) for 60 min, which was further amplified by an anti-rabbit secondary antibody. All steps were performed in a humidity chamber at 20 °C. A biotinylated polyvalent anti-rabbit secondary antibody and then streptavidin-peroxidase enzyme (Shandon, Pittsburg, USA) were placed on sections, each for 10 min. For color reaction, 3-amino-9-ethylcarbozole (AEC) chromogen (Shandon, Pittsburg, USA) was applied to the sections for 10-15 min (controlled by visual observation under a microscope). Sections were rinsed with distilled water, counterstained with Gill's hematoxylin for 1-2 min, and mounted with aqueous mounting medium. The same procedure, except placing primary antibody on sections, was applied to the sections prepared from the tissue of the control animals.

Results

Clinical findings

Loss of appetite, ruffling feathers, labored breathing, a tendency to separate from healthy birds, and green to greenish-yellow diarrhea were observed in the 1-day-old group between days 3 and 9 pi. Chalky white feces adhered to the area around the cloaca. All poults exhibited somnolence between days 6 and 9 pi. No clinical signs were evident in the other groups, except for diarrhea (Figure 1A).

Gross lesions

In the 1-day-old group yellowish-grey foci (1-2 mm in diameter) were observed in the dorsal and ventral lobes of the lungs on days 5-13 pi. Petechial hemorrhages were present on the parietal surface of the liver. In the 3-week-old group, yellowish, miliary, and diffuse foci were seen on the parietal and visceral surfaces of the liver on day 7 pi. These foci ranged from 1-5 mm in diameter and exuberated to the outer surface of the liver in 1 animal in the 2-month-old group (Figure 1B). Similar lesions were observed in the serosa of the intestines and epicardial surface. Spleens were enlarged, congestive with necrotic foci, and yellowish in all of the treatment groups. In some cases in the 2-month-old group, ulcers disseminating from the duodenum to caecum were present. In all of the treatment groups the subserosal vessels of the intestines, especially the caecal ones, were congested. Involution of the bursa of Fabricius and hemorrhaging in the lungs were observed.

Histopathological lesions

Lesions were observed between days 3 and 15 pi in the 1-day-old group, in which severe hyperemia and hemorrhaging were observed in the lungs. In some areas severe lymphoid cell infiltration was evident in the lumens of the tertiary bronchi, and normal structures of the bronchi had disappeared. On day 10 of infection, heterophilic granulomas with coagulation necrosis in the centers that contained degenerated heterophils and were surrounded by multinucleated giant cells were seen in the lungs. Diffuse fat accumulation and passive hyperemia were evident in the liver in the 1-day-old group. These lesions were also observed on days 5-12 pi in the 3-week-old group and on days 7-18 pi in the 2-month-old group. Heterophilic granulomas were present in

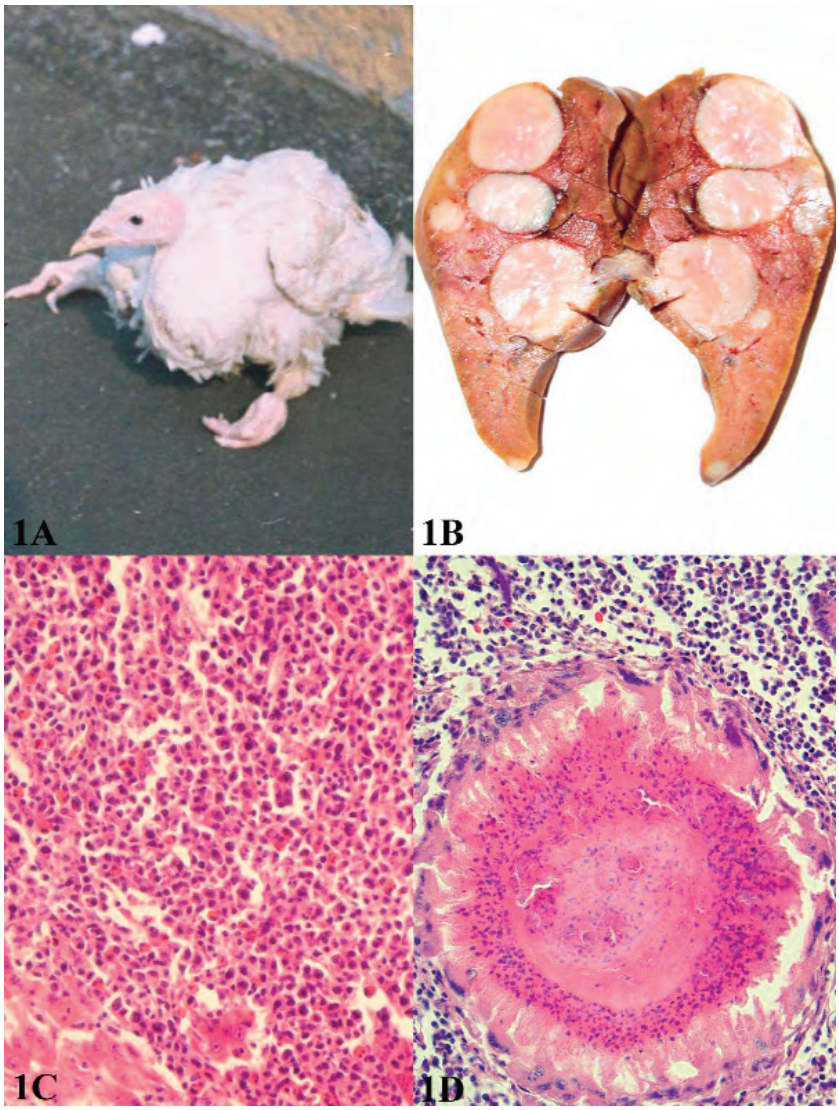

Figure 1 A. Clinical appearance of a poult in the 2-month-old group.
B. Yellowish nodular foci in the liver of a 2-month-old poult.
C. Cell infiltration with heterophils, as well as histiocytes, in the liver of a 2-month-old poult (H&E 400×).
D. Granulomas with heterophil and lymphoid cell infiltration in the submucosa of the duodenum of a 2-month-old poult (H&E 400×).

the livers on day 7 pi in the 3-week-old group versus on day 12 pi in 2-month-old group. In the 2-month-old group large nodular foci disseminated to virtually all the parenchyma, along with heterophils and histiocytes in the liver were observed in 1 poult (Figure 1C). In some areas of the spleen disseminated coagulation necrosis was present on almost all parenchyma in 15 of the 20 poults in the 1-day-old group. The same lesions were detected in all of the animals in the 3-week-old and 2-month-old groups, and lymphocytes were absent in the lymphoid follicles and a reticular structure became apparent in these 2 groups. In the kidneys of the poults in 3-week-old and 2-month-old groups tubular epithelial cells exhibited degenerative changes in many parts, while some were completely necrotic. Histiocytic-type cell infiltrations,

including lymphocytes, were observed primarily in the intertubular area and in some parts of the cortex. Severe histiocytic cell infiltrations were present over most of the pancreatic parenchyma, as well as in the myocardium of the poults in the 2-month-old group. In addition, severe heterophil, lymphocyte, and macrophage infiltration was seen between the muscle fibers in 1 patient in the 2-month-old group. In all groups, cystic, degenerative, and necrotic changes that ranged from mild to moderate in severity were observed in the follicles and in the mucosal epithelium of the bursa of Fabricius. Heterophil infiltration was seen in some parts of the follicular areas in some of the poults in the 2-month-old group. In all the groups lymphoid cell infiltration of varying severity was observed in the propria and submucosa of the intestines, especially in the caecum, and necrotic changes were also seen in other parts of the intestines. Granulomas accompanied by heterophil and lymphoid cell infiltration were evident in all sections of the intestine of 1 poult in the 2-month- old group (Figure 1D). Necrotic changes and cystic dilatation were observed in the epithelium of the cloacal gland in the poults in all 3 treatment groups. No clinical, macroscopic, or histopathological findings were observed in the age-matched control groups.

Bacteriological findings

S. Gallinarum was isolated on days 1-15 pi in the poults in all 3 treatment groups (Table).

Immunohistochemical findings

The bacterial antigen of *S.* Gallinarum strain 9 was detected in the macrophages of the propria mucosa of the bronchi and in the epithelium of the mesobronchi of lung samples collected on days 4 -14 pi in the 1-day-old group (Figure 2A).

Strong immunoreactivity was noted in the cytoplasm of epithelial cells of the mucosa of the ileum, caecum (Figure 2B), and bursa of Fabricius, while immunolocalization was weak inside and outside of necrotic cells located in the propria mucosa of these tissue samples on days 4-10 pi in the 3-week-old group and on days 16-18 pi in the 2-month-old group (Figure 2C).

Immunoreactivity was strong in the epithelial cells of the duodenum, jejunum, and cloacal gland (Figure 2D). In the liver and spleen, weak extra-cellular immunoreactivity was noted between and around the pyknotic cells, while weak intra-cellular immunoreactivity was observed in the granulomas in the liver. No antigen was detected in the tissue samples collected from the poults in the control groups.

Discussion

In the present study, clinical, pathological, immunohistochemical, and bacteriological findings associated with experimentally induced *S.* Gallinarum strain 9 infection in white turkey poults were evaluated. Because *S.* Gallinarum strain 9 is pathogenic to all turkey age groups (1,7,15), the poults were divided into 3 treatment groups based on age: 1-day-old, 3-week-old, and 2-month-old. It has been reported that infection caused by *S.* Gallinarum strain 9 results in high morbidity and that mortality ranged from 10% to 90% (4). In turkeys, mortality may be as high as in chickens (1). Hinshaw (16) reported a mean mortality of 26.5%. In contrast, in the present study there was no mortality in any of the treatment groups, which may have been due to the resistance of white turkeys to *S.* Gallinarum infection, as well as to low virulence of the agent. In the acute form of infection, labored breathing, loss of appetite, ruffling of feathers, a tendency to separate from healthy birds, and green to greenish-yellow diarrhea are characteristic signs (17-19). In the present study these characteristic findings were observed in the 1-day-old group, whereas no clinical signs, except for greenish-yellow diarrhea, were observed in the 3-week-old and 2-month-old groups, which may have been due to the pathogenicity of the agent and undeveloped immune systems in the 1-day-old poults and a decrease in the appearance of clinical symptoms with age. Lameness was reported as a common finding in the infection caused by *S.* Pullorum in some flocks (20).

In the present study gross lesions that were observed in the acute form of *S.* Gallinarum infection in turkey poults were similar to the lesions seen in chickens that were reported by Shivaprasad (1). In the chronic form of the infection in chickens, proliferative lesions are seen in the heart, intestines, pancreas, and liver (19,21,22). In the present study similar findings

Table. The comparative findings of bacteriological and immunohistochemical examinations in turkey poults infected with *Salmonella* Gallinarum strain 9.

Days[a]	Groups[b]	Organs																	
		Lung		stomach		bm		spleen		bF		int		kidney		heart		cs	
		BEc	IHEd	BE	IHE	BE	IHE	BE	IHE	BE	IHE	BE	IHE	BE	IHE	BE	IHE	BE	IHE
1	1[e]	-	+	+	-	-	-	+	+	+	+	+	+	-	-	+	-	-	nd
	2[e]	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	-	+	nd
	3[e]	+	-	+	-	-	-	+	+	+	-	+	+	+	+	+	-	+	nd
2	1[e]	-	+	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	nd
	2[e]	+	+	-	-	-	-	+	+	+	+	+	+	+	-	+	-	+	nd
	3[e]	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	nd
3	1[e]	-	+	-	+	+	-	-	+	-	-	+	+	+	-	-	-	-	nd
	2[e]	+	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	-	nd
	3[e]	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	nd
4	1[e]	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	nd
	2[e]	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	nd
	3[e]	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	nd
5	1[e]	+	+	+	-	-	-	+	+	+	+	+	+	-	-	+	-	+	nd
	2[e]	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	nd
	3[e]	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-	+	nd
6	1[e]	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	-	-	nd
	2[e]	-	+	-	+	-	-	+	+	+	+	+	+	+	-	+	-	+	nd
	3[e]	-	-	+	+	+	-	-	+	+	+	+	+	+	-	+	-	+	nd
7	1[e]	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	+	nd
	2[e]	+	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+	nd
	3[e]	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	nd
8	1[e]	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	nd
	2[e]	+	-	+	-	-	-	+	+	-	+	-	+	+	+	-	-	-	nd
	3[e]	-	-	+	+	-	-	+	+	+	+	+	+	-	-	-	-	+	nd
9	1[e]	+	+	-	+	-	-	+	+	+	+	-	-	-	-	+	-	+	nd
	2[e]	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	nd
	3[e]	+	+	+	-	-	-	-	+	+	+	+	-	+	+	-	+	-	nd
10	1[e]	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	nd
	2[e]	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	+	+	nd
	3[e]	-	+	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+	nd

Table. (Continued).

11	1[e]	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	nd
	2[e]	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	nd
	3[e]	-	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	nd
12	1[e]	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-	-	nd
	2[e]	+	-	-	+	-	-	+	+	-	-	-	+	-	-	-	+	-	nd
	3[e]	-	-	-	+	+	-	+	+	-	+	-	+	+	+	-	+	-	nd
13	1[e]	+	+	-	+	-	-	+	+	-	+	-	+	-	-	+	+	+	nd
	2[e]	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	nd
	3[e]	-	-	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	nd
14	1[e]	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	nd
	2[e]	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	nd
	3[e]	-	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+	nd
15	1[e]	+	-	+	+	-	-	+	+	+	+	-	+	-	-	+	+	+	nd
	2[e]	-	-	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	nd
	3[e]	-	-	-	-	+	+	+	+	-	+	+	-	+	+	-	-	-	nd
16	1[e]	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	nd
	2[e]	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	nd
	3[e]	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	nd
17	1[e]	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	nd
	2[e]	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	nd
	3[e]	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	nd
18	1[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd
	2[e]	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	nd
	3[e]	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	nd

[a]: Days of organ collection after inoculation, [b]: groups (1: 1- d- old, 2: 3-wk-old, 3: 2 –month-old), [c]: Bacteriological examination,
[d]: Immunohistochemical examination, [e]: two animals for each groups.
[+]: Positive [-]: Negative bm: bone marrow, bF: bursa of Fabricius int: intestine, cs: cloacal swab nd: not done

were observed in the liver, heart, kidneys, and pancreas in 2-month-old poults, while they were restricted to liver and kidneys in 3-week-old poults. Unlike chickens, ulcers disseminating from the duodenum to caecum are the common lesions in turkeys (1). Observation of similar ulcers in some of the 2-month-old poults in the present study may suggest that breed is also an important factor in the development of such lesions, as well as age. In the mucosal epithelium of the bursa of Fabricius, small cystic, degenerative, and necrotic changes in the 1-day-old group, and heterophil infiltration in the parafollicular area and in the centers of the follicles in the 2-month old-group were observed in the present study; however, to the best of our knowledge this is the first study to report the development of lesions in the bursa of Fabricius in *S.* Gallinarum-infected turkeys. In the present study, in turkey poults, presence of similar (8) but slighter lesions than those seen in the bursa of Fabricius of the chicken may suggest that this organ is less sensitive to the agent.

Gram-negative bacteria stimulate acute heterophilic and chronic granulomas in poultry species (23). Granulomas caused by *Salmonella*

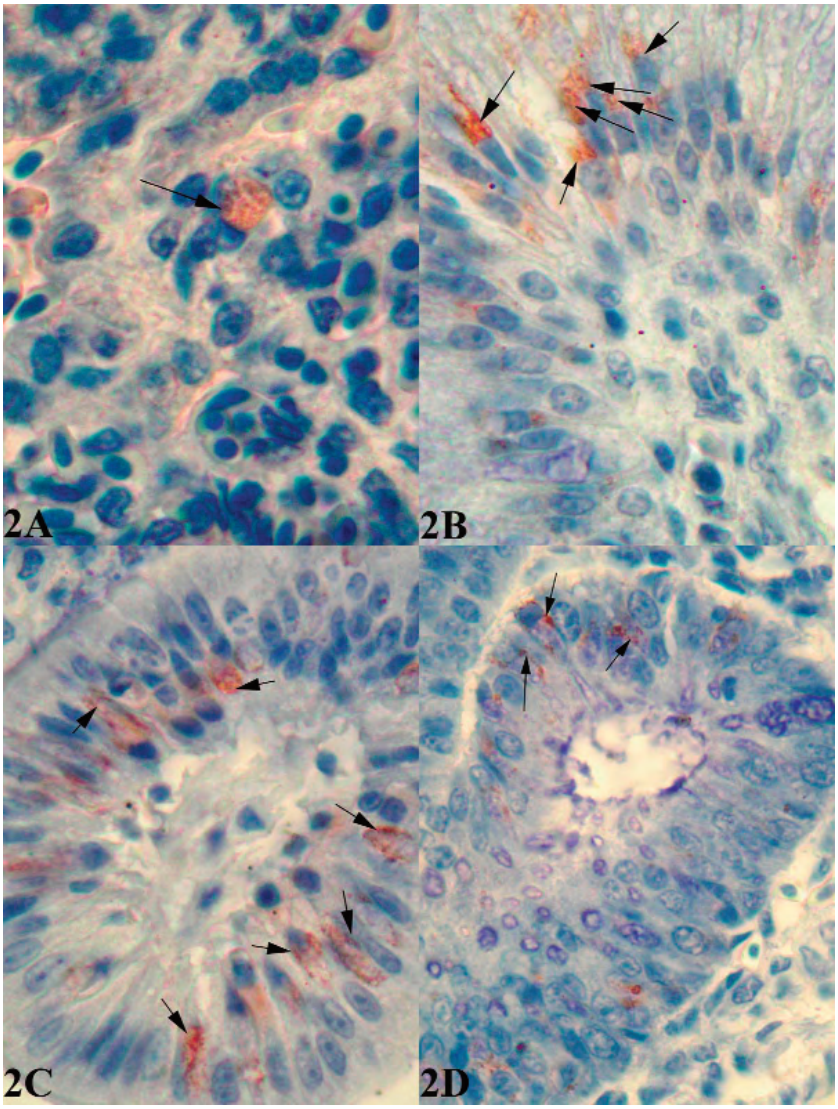

Figure 2 A. *S.* Gallinarum positive reaction in the cytoplasm of macrophage in the lungs of 1-d-old poult (arrows), (immunoperoxidase/hematoxylin 1000×).

B. *S.* Gallinarum positive reaction in the cytoplasm of epithelial cells of caecal villus of 1-d-old poult (arrows), (immunoperoxidase/hematoxylin 1000×).

C. *S.* Gallinarum -positive reaction in the cytoplasm of mucosal epithelial cells of the bursa of Fabricius in a 1-day-old poult (arrows) (immunoperoxidase/hematoxylin, 1000×).

D. *S.* Gallinarum -positive reaction in the cytoplasm of glandular epithelial cells of the cloaca of a 3-week-old poult (arrows) (immunoperoxidase/hematoxylin, 1000×).

species were detected in the lungs (8), liver, and caecum (12,22). In the present study observation of heterophilic granulomas in the lungs in the 1-day-old group, in the liver in the 3-week-old group, and in the duodenum of the 2-month-old group shows that *S.* Gallinarum also caused granulomas in the turkey poults and that these granulomas have also affinity to duodenum.

Immunohistochemical techniques are of great importance in the rapid differential diagnosis, because they identify the definitive location of the agent in tissues (9-11,24); however, to the best of our knowledge, ours is the first study to use immunohistochemical techniques in the diagnosis of *S.* Gallinarum in turkeys. Immunoreactivity was observed from days 1-18 pi immunohistochemically, while the agent was isolated from days 1-15 pi using bacteriological methods. Detection of the agent immunohistochemically for a longer period of time than bacteriologically shows that immunohistochemical detection is possible whether or not the bacteria are alive, whereas the bacteria must be alive for detection in culture.

The results of the present study have shown that white turkey poults were slightly susceptible to infection caused by *S.* Gallinarum. *S.* Gallinarum infection caused heterophilic granulomas, especially in the duodenum of turkey poults, as well as cystic, degenerative, and necrotic changes in the bursa of Fabricius. Immunohistochemical analysis can be considered a useful tool for detecting *S.* Gallinarum at various phases of infection and it can be used as an adjunct to bacteriological examination in the diagnosis of *S.* Gallinarum.

Acknowledgements

The authors thank Dr. Paul Barrow (Animal Health Compton Laboratory, UK) for supplying the *S.* Gallinarum strain 9.

References

1. Shivaprasad, H.L.: Pullorum disease and fowl typhoid. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E., Eds., Diseases of Poultry, 11th edn. Iowa, USA: Iowa State Press. 2003; 568-582.
2. Shivaprasad, H.L.: Fowl typhoid and pullorum disease. Rev. Sci. Tech. Off. Int. Epiz., 2000; 19: 405-424.
3. Barrow, P.A.: Salmonella control-past, present and future. Avian Pathol., 1993; 22: 651-669.
4. Williams Smith, H., Tucker, J.F.: The virulence of Salmonella strains for chickens: their excretion by infected chickens. J. Hyg. (Lond), 1980; 84: 479-488.
5. Humbert, F., Salvat, G.: The risk of transmission of Salmonellae in poultry farming: detection and prevention in Europe. Rev. Sci. Tech. Off. Int. Epiz., 1997; 16: 83-90. (article in French with an abstract in English)
6. Mead, G.C.: Prospects for 'competitive exclusion' treatment to control Salmonellas and other food borne pathogens in poultry. Vet. J., 2000; 159: 111-123.
7. Arora, A.K., Gupta S.C., Kaushik, R.K.: Isolation of *Salmonella gallinarum* from turkeys. Ind. Vet. J., 1988; 65: 171.
8. Beyaz, L., Kutsal, O.: Pathological and immunohistochemical studies in experimental Salmonella gallinarum infection (fowl typhoid) in chickens. Ankara Üniv. Vet. Fak. Derg., 2003; 50: 219-227. (article in Turkish with an abstract in English)
9. McRill, C.M., Kramer, T.T., Griffith, R.W.: Application of the peroxidase-antiperoxidase immunoassay to the identification of Salmonellae from pure culture and animal tissue. J. Clin. Microbiol., 1984; 20: 281-284.
10. Porter, R.E. Jr., Holt, P.S.: Effect of induced molting on the severity of intestinal lesions caused by *Salmonella enteritidis* infection in chickens. Avian Dis., 1993; 37: 1009-1016.
11. Pospischil, A., Wood, R.L., Anderson, T.D.: Peroxidase-antiperoxidase and immunogold labeling of *Salmonella typhimurium* and *Salmonella choleraesuis* var *kunzendorf* in tissues of experimentally infected swine. Am. J. Vet. Res., 1990; 51: 619-624.
12. Desmidt, M., Ducatelle, R., Haesebrouck, F.: Pathogenesis of *Salmonella enteritidis* phage type four after experimental infection of young chickens. Vet. Microbiol., 1997; 56: 99-109.
13. Luna, L.G.: Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. 3rd edn., McGraw-Hill, New York. 1968.
14. Arda, M., Minbay, A., Aydın, N., Akay, Ö., İzgür, M.: Kanatlı Hayvan Hastalıkları. 2. Baskı. Medisan Yayınevi, Ankara. 1994; 76-78.
15. Hewitt, E.A.: Bacillary white diarrhea in baby turkeys. Cornell Vet., 1928; 18: 272-276.
16. Hinshaw, W.R.: Fowl typhoid on turkeys. Vet. Med., 1930; 25: 514-517.
17. Kokosharov, T., Hristov, H., Belchev, L.: Clinical, bacteriological and pathological studies on experimental fowl typhoid. Indian Vet. J., 1997; 74: 547-549.
18. de Oliveria, G.H., Junior, A.B., Fernandes, A.C.: Experimental infection of laying hens with *Salmonella enterica* serovar Gallinarum. Braz. J. Microbiol., 2005; 36: 51-56.

19. Smith, H.W.: Observations on experimental fowl typhoid. J. Comp. Pathol., 1955; 65: 37-54.

20. Salem, M., Odor, E.M., Pope, C.: Pullorum disease in Delaware roasters. Avian Dis., 1992; 36: 1076-1080.

21. Kaushik, R.K., Singh, J., Kumar, S., Kulshreshtha, R.C.: Fowl typhoid in a few poultry farms of Haryana state. Indian J. Anim. Sci., 1986; 56: 511-514.

22. Sato, Y., Kumeta, A., Koyama, T., Takada, T., Aoyagi, T., Ichikawa, K., Wada, K., Furuya, T., Tanaka, K.: An outbreak of *Salmonella thyphimurium* infection in bengaless, a variety of Lonchura striata. J. Vet. Med. Sci., 1993; 55: 1073-1076.

23. Montali, R.J.: Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). J. Comp. Pathol., 1988; 99: 1-26.

24. Hoop, R.K., Pospischil, A.: Bacteriological, serological, histological and immunohistochemical findings in laying hens with naturally acquired *Salmonella enteritidis* phage type 4 infection. Vet. Rec., 1993; 133: 391-393.