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ANTIGENIC VARIATIONS BETWEEN DIFFERENT SALMONELLA SEROTYPES ISOLATED FROM CHICKENS

(With 6 Tables and 2 Photos)

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الاختلافات الأنتيجينية بين عترات السالمونيلا المختلفة المعزولة من الدجاج

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باجراء الفحص البكتريولوجى لعينات مختلفة من ٧٥٠ حالة مرضية من الدجاج فى مختلف مراحل النمو أمكن عزل ٤٢ معزولة من السالمونيلا (٥,٧٥ %). وعند التصنيف السيرولوجى لمعزولات السالمونيلا تبين أنها تنتمى الى سالمونيلا انتريتيدس (٢٠ معزولة) , سالمونيلا تيفيميوريم (١٣ معزولة) , سالمونيلا انفانتيس (٥ معزولات) , سالمونيلا مونتيفيديو (٣ معزولات) وسالمونيلا سيرو (معزولة واحدة). وأوضحت اختبارات الحساسية المعملية لمعزولات السالمونيلا المختلفة تعدد مقاومة كل منها لتأثيرواحد أو أكثر من مضاد حيوى من المضادات الحيوية المستخدمة. كذلك أثبتت اختبارات العدوى الأصطناعية للعترات المختلفة فى الكتاكيت عمر يوم ضراوتها جميعا بدرجات متفاوتة باختلاف نوع العترة وكذلك ثبت ايجابية العترات المعزولة لاختبار الإرتباط بصبغة الكونجو الحمراء وذلك للتمييز بين العترات الممرضة والغير ممرضة. وقد أوضح التحليل الكهربائى للعترات المختلفة باستخدام طريقة س د س فصل ٦-١٠ حلقات بروتينية مختلفة تتراوح بين ١٣,٦٣١ الى ٢٠٠,٥ كيلو دالتون وذلك حسب نوع العترة. كما أوضح اختبار الطبع المناعى لبروتينات الغشاء الخارجى لميكروبات السالمونيلا أنها تشترك فى العديد من الحلقات ذات الخصائص المناعية عند ١٥, ٢٩, ٣٥ ك.د. وكانت أعلى الحلقات البروتينية المناعية للسالمونيلا تيفيميوريم عند ٣٥,٤ ك.د., بينما كانت في السالمونيلا انتريتيدس عند ٢٩ ك.د., وفى السالمونيلا مونتيفيديو عند ٣٩,٨ ك.د., وفى السالمونيلا انفانتيس عند ٢٥,١٢٨ك.د , ٣٤,٠٩١ ك.د. بينما فى السالمونيلا سيرو كانت أعلى حلقة بروتينية مناعية عند ١٤٣,٠٨ ك.د. وفى المجمل, فقد ثبت أن استخدام كل من التحليل الكهربائى واختبار الطبع المناعى يعتبر من التقنيات الحديثة التى تتيح التشخيص السريع والدقيق للاصابة بالسالمونيلا فى الدجاج وكذلك يمكن من خلالها التعرف على الحلقات الأنتيجينية لعترات السالمونيلا المختلفة مما يمكن من أستخدامها فى أنتاج لقاح للوقاية من الاصابة بالسالمونيلا فى الدجاج.

SUMMARY

Examination of different samples from 750 chicken cases of various growth stages, revealed the isolation of 42 Salmonella isolates (5.75%). The isolated salmonellae were serotyped as *Salmonella enteritidis* (20 isolates), *Salmonella typhimurium* (13 isolates), *Salmonella infantis* (5 isolates), *Salmonella montivideo* (3 isolates) and *Salmonella cerro* (1 isolate). Antibiogram of the isolated Salmonellae indicated multidrug-resistance to one or more than of the tested antimicrobial agents. Pathogenicity tests in one-day old chicks proved the virulence of all examined serovars with various degrees of pathogenicity and all were positive for congo red activity. SDS-PAGE protein analysis of different serovars revealed 6-10 protein bands ranged from 13.631-200.5 KD, which in relation to the isolated Salmonella serovar. Immunoblotting of the isolated serovars revealed the presence of common protein bands at 15, 29 and 35 KD. The highest antigenicity protein band of *S. typhimurium* was detected at 35.4 KD, while in *S. enteritidis* was detected at 29KD; in *S. montivideo* at 39.8 KD; in *S. infantis* at 25.128 and 34.091 KD, while *S. cerro* had the highest antigenic protein band at 143.08KD. In conclusion, SDS-PAGE analysis and immunoblot provide a recent and accurate techniques for detection of salmonellosis in chickens, in addition to offer the use of immunogenicity of different detected immunogenic bands to serve as components of an effective subcellular vaccine for poultry salmonellosis.

Key words: *Chickens, Salmonella, antimicrobial agents*

INTRODUCTION

In the last decades, poultry and poultry products have been the main source of non-host specific Salmonella infecting humans (Shahata, 1979, Abd El-Hamid *et al.,* 2004 and Murugkar *et al.,* 2005). Poultry are commonly infected with a wide variety of Salmonella serovars and there has been considerable variation in the occurrence of the most common Salmonella serovars in domestic fowls in different countries and at different times.The outer membrane protein analysis has proved to be useful technique in characterization of Salmonella (Fadl *et al.,* 2002 and Ochoa-Reparz *et al.,* 2004). On the practical basis, detection of flock infections remains one of the most serious unsolved problems in controlling salmonellosis in poultry. Serological studies of different salmonellae revealed the presence of cross reaction between Salmonella

organisms and other members of family Enterobacteriaceae such as *Escherichia coli* and *Proteus spp*., due to the presence of a common antigen (Le-Minor *et al.,* 1982). Thus, increased interest for the control of salmonella in poultry requires the development of improved detection methods. Western immunoblotting is a convenient, sensitive specific technique for the detection of antigen and antibodies (Kim and Nagaraja, 1991). The purpose of this research work was to determine the incidence of different Salmonella serovars isolated from chickens at different stages of growth with reference to their protein analysis using SDS-PAGE as well as to study the virulence pattern of different serovars in relation to the antigenic variation between them using immunoblotting technique.

MATERIALS and METHODS

Chicken specimens:

Samples from liver, spleen, intestines (ceci and cecal tonsils), yolk sacs and bone marrows were collected under complete aseptic conditions from 750 chicken cases at different growth stages (chicks, broilers and parents) either dead (180 cases) or living ailing (550 cases) in the period from January 2006 up to March 2007. The samples were submitted to Bacteriological Unit of the Department of Diagnosis and Research of Poultry Diseases, Animal Health Research Institute, Dokki, Giza, Egypt, to be examined bacteriologically for the isolation of different salmonellae.

Isolation and identification of Salmonella from chickens

Isolation of different salmonellae from chicken samples was carried out according to Mallinson and Snoeyenbos (1994). Suspected colonies were identified morphologically, culturally and biochemically according to Holt *et al.,* 1996 and Collier *et al.,* 1998). Serological identification was carried out by slide agglutination test using polyvalent and monovalent [O] and [H] Salmonella antisera according to Kauffmann-White scheme described by Kauffmann (1974).

Antibiogram of the isolated salmonellae: In-vitro susceptibility testing of Salmonella isolates to various antimicrobial agents was determined using NCCLS method (NCCLS, 2003). Ten different commercial antibiotic discs (Oxoid) were used.

Pathogenicity tests of the isolated Salmonella serovars:

1-Pathogenicity in one-day old chicks:

Six groups (1 to 6) each of twenty, one-day old chicks which proved to be salmonella–free, were used for pathogenicity testing of various isolated Salmonella serovars. Each group was divided into two

sub-groups A and B. Chicks in sub-groups A were infected orally with $3X10^8$ CFU (colony forming units) of one of the isolated serovars namely: *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella infantis*, *Salmonella montivideo* and *Salmonella cerro*. Chicks in subgroups B were inoculated intrapretoneally with one of the same serovars at the same dose, while the 6^{th} subgroups were given 0.5 ml of sterile saline solution orally and intrapretoneally, respectively and were saved as non infected control. All chicks in different groups were kept separately and monitored for clinical signs and mortality for 14 days post-infection (Bakshi *et al.,* 2003). Postmortem examinations accompanied by re-isolation of the infected microorganisms from internal organs of dead chicks in different groups were attempted.

2- Congo red (CR) test: All Salmonella isolates were tested for its growth status on congo red medium modified according to Berkhoff and Vinal (1986).

SDS-PAGE technique: Salmonella antigens were prepared after Ahmed *et al.,* (1998). Protein of various Salmonella serovars was separated by SDS-PAGE using the discontinuous buffer system described by Laemmli (1970).

Western blotting:

A- Preparation of Salmonella antisera: New Zealand white rabbits were used to produce antisera against different isolated salmonella serovars according to Kim and Nagaraja (1991).

B- Application of Western immunoblotting: To identify proteins specific for each of the isolated Salmonella serovars, Western blotting was done using the prepared salmonella antisera according to the procedure described by Talbot *et al.,* (1984) The outer membrane proteins (OMPs) of different salmonella serovars were separated by SDS-PAGE by the method described by Laemmli (1970) and were electrophoretically transferred to a nitrocellulose filter using transphor electrophoresis unit cell with electroblotting buffer containing 25mM Tris, 192 mM glycine, and 20% methanol, pH 8.3. The nitrocellulose filter strips were stained by Ponceau S red staining (Sigma), to check the transferred proteins and destained in distilled water. The strips were then immersed in Tris-buffered saline (TBS) containing 20 mM Tris, 500 mM NaCl, and 3% gelatin, pH 7.5, for 1hour at 37˚C. The nitrocellulose filters were rinsed briefly in Tris-Tween buffer saline (TTBS) containing 0.05% Tween-20 in TBS buffer, pH 7.5. The resulting blots were incubated for 3 hours at 37˚C with serum containing rabbit anti-Salmonella antibodies of the corresponding Salmonella serovar diluted

in TTBS containing 1% gelatin. The unbound antibodies were removed by rinsing the blot in TTBS and the bound antibodies were detected by using conjugate labeled with horseradish peroxidase (HRP) and substrate 4 chlor-1-naphthol (Sigma).

RESULTS

Table 1: Prevalence of Salmonella isolation from chickens.

Examined cases		Salmonella positive	
Type	Number	Number	%
Living ailing	550	33	6
Dead	180	9	5
Total	750	42	5.75

Table 2: incidences, Congo red activity and antigenic structures of Salmonella serovars isolated from chickens

Salmonella Serovar	Total		Congo red activity		Sero-group	Antigenic structure	
	No.	%	Positive	Negative		[O]	[H]
S.enteritidis	20	47.62%	20	0	D1	1,9,12	g,m
S.typhimurium	13	30.95%	13	0	B	1,4,5,12	i:1,2
S.infantis	5	11.90%	5	0	C1	6,7	r:1,5
S.montivideo	3	7.14%	3	0	C1	6,7	g,m,s,p
S.cerro	1	2.38%	1	0	K	6,14,18	Z4,Z28:1,5
Total	42	100%	42	0			

Table 3: Antibiogram of salmonellae isolated from chickens.

Antimicrobial agents	Disc conc. (µg)	Antibiotic susceptibility test		
		Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	25	9/42(21.4%)	0/42(0%)	33/42(78.6%)
Amoxicillin	20	0/42(0%)	5/42(11.9%)	37.42(88.1%)
Cephridin	30	31/42(73.8%)	11/42(26.2%)	0/42 (0%)
Chloramphenicol	30	15/42(35.7%)	2/42(4.7%)	25/42(59.5%)
Ciprofloxacin	10	42/42(100%)	0/42(0%)	0/42(0%)
Danofloxacin	10	42/42(100%)	0/42(0%)	0/42(0%)
Norfloxacin	10	42/42(100%)	0/42(0%)	0/42(0%)
Gentamycin	10	14/42(33.3%)	5/42(11.9%)	23/42(54.7%)
Tetracycline	30	0/42(0%)	3/42(7.1%)	39/42(92.9%)
Sulphamethoxazole-trimethoprim	25	31/42(73.8%)	0/42(0%)	11/42(26.2%)

Table 4: Pathogenicity tests of isolated Salmonella serovars in one-day old chicks.

GP. NO.	Salmonella serovar	Sub-group	Route of infection	Number of dead chicks /day*													Total	
				1	2	3	4	5	6	7	8	9	10	11	12	13	NO.	%
1	*S.entiritidis*	A	Oral	0	0	2	2	0	1	0	1	0	0	1	1	0	8	80
		B	I/P	7	3	0	0	0	0	0	0	0	0	0	0	0	10	100
2	*S. typhimurium*	A	Oral	0	0	2	2	2	1	0	1	0	1	0	0	0	9	90
		B	I/P	9	1	0	0	0	0	0	0	0	0	0	0	0	10	100
3	*S. infantis*	A	Oral	0	0	2	1	1	1	1	0	0	0	0	1	0	7	70
		B	I/P	6	4	0	0	0	0	0	0	0	0	0	0	0	10	100
4	*S.montivideo*	A	Oral	0	0	0	1	2	2	0	1	1	1	0	0	0	8	80
		B	I/P	7	3	0	0	0	0	0	0	0	0	0	0	0	10	100
5	*S.cerro*	A	Oral	0	0	0	1	1	1	2	2	0	1	1	1	0	7	70
		B	I/P	8	2	0	0	0	0	0	0	0	0	0	0	0	10	100
6	Control negative	A	Oral	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		B	I/P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NO.= number I/P=Intraperitoneal *=days post-infection

Table 5: The molecular weights (Mol.wt.) of Salmonella serovars compared with the molecular weights of the Sigma marker

Lanes:	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
Bands	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
1	250	180.88	200	200	200.5	200.33
2	160		150.5	180.3	150.9	150
3	130	120.3				
4	105		100.66		100	100.25
5	85					
6	75		75.1	75.00		75.4
7	66	60.15	62.5	62.112		62.71
8	50			.		
9	35	35.00	35.00	34.00	35.00	36.532
10	24	26.00	25.721	25.66	25.00	25.921
11						
12	18.4		19.095	18.559		
13	.	15.471	15.81	15.643	15.532	15
14	14.3		14.5			13.631

Lane (1): protein marker Lane (2): *S.cerro* Lane (3): *S.enteritidis* Lane(4): *S.infantis* Lane (5): *S.montivideo* Lane (6): *S.typhimurium*

Table 6: The amount of protein and molecular weight of bands of western blot of Salmonella species compared with the molecular weights of western blot of bio lab-broad range prestained molecular weights marker

Lanes:	Lane 1 *S.t yphimurium*		Lane 2 *S.cerro*		Lane 3 *S.enteritidis*		Lane 4 *S.montivideo*		Lane 5 *S.infantis*		Lane 6 marker	
Bands	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)
1	147.31	6.1287	143.08	12.483	103.13	8.7253	138.85	22.82	97.5	2.7242	250	9.1327
2	70.968	8.1718	59.677	6.7738							160	19.524
3	43.8	3.0548	43	9.7839							105	21.988
4	42.2	6.7024			41.4	3.619	41.8	3.8294	40.2	34.197	75	9.1018
5	39	2.0458	39.2	2.4591	39.6	6.2596	39.8	12.591	34.945	2.5488	50	12.279
6	35.4	34.717	35.788	7.04	34.924	1.6107	35.194	2.7216	34.091	38.626		
7	33.41	3.337	33.273	4.3285	33.409	5.4027	33.12	1.9114	33.333	3.3596	35	9.5337
8	29.12	13.222	29.3	5.5432	29	11.651	29.421	11.038	29.11	12.097	30	7.4348
9	27.179	4.5188	27.051	3.1935	27	2.7661	27	0.47963	25.128	68.032	25	21.37
10	23.793	5.8539	23.276	6.5721	23.032	8.5662	23.1	3.9863	23.119	7.521		
11	19.31	24.97	18.276	7.744	17.759	6.0615	18.103	4.1006				
12	16.552	29.288	16.034	8.1082								
13	15	31.448	15	4.2871	15	9.3732	15	6.8058	15.345	12.578	15	9.0185
14					11.875	6.9632	11.875	12.288	13.125	17.064	10	10.736

Photo 1: SDS-PAGE protein profile of Salmonella serovars isolated from chickens.

Lane (1): protein marker, Lane (2*): S. cerro*, Lane (3): *S. enteritidis*, Lane (4): *S. infantis*, Lane (5): *S. montevideo*, Lane (6): *S. typhimurium*

Photo 2: Western immunoblot of Salmonella serovars isolated from chickens.

Lane (1): *S.typhimurium*, Lane (2): *S.cerro*, Lane (3): *S.enteritidis*, Lane(4):*S.montevideo*, Lane (5): *S.infantis*, Lane(6): protein marker

DISCUSSION

Bacteriological examination of different samples obtained from chicken cases at different ages revealed that out of 730 examined cases 42 Salmonella isolates were obtained with an incidence of 5.75% (Table 1).

Similar results were obtained by Hassan *et al.,* 2003 who isolated 35 Salmonella isolates (5.51%) out of 635 examined chicken samples. On serotyping of the isolated Salmonellae (Table 2) revealed that *Salmonella enteritidis* was the most predominant isolated serovar (47.62%) followed by *Salmonella typhimurium* (30.95%). These results agreed with that obtained by Abd-Allah *et al.,* (1995), who surveyed a large number of samples from different domestic birds and their environmental surroundings in El-Fayoum governorate and found that *Salmonella enteritidis* was the most prevalent isolated serovar (40%) followed by *Salmonella typhimurium* (24%), *Salmonella montivideo* (16%). However, it was noticed that during the last 10-15 years, *Salmonella enteritidis* has replaced *Salmonella typhimurium* as the commonest serovar in many countries worldwide (Poppe, 2000). Isolates of *Salmonella infantis* constituted 11.9% of the total number of Salmonella isolates which agreed with the results obtained by Novak and Polaharova (1993) and Hassan *et al.,* (2003). *Salmonella montivideo* represented 7.14% of the isolated salmonellae, while *Salmonella cerro* constituted 2.38%. the same serovars were isolated from poultry samples with different incidences by many authors (Barnhart *et al.,* 1992, Abd-Allah 1995 and Hofer *et al.,*1998).

Concerning Congo red binding activity, all the isolated Salmonellae were proved to be Congo red positive. It was reported that Congo red binding activity was correlated to the invasiveness of bacteria, as fimbria promote the binding of the hydrophobic dye Congo red by the bacteria that produce such fimbria (Qadri *et al.,* 1988). Thus, Congo red binding test may provides a simple and rapid test for screening Salmonella strains which harbor fimbria (Dorn *et al.,* 1992).

Antibiogram of the isolated Salmonellae (Table 3) revealed multi-resistant to more than one of the tested antibiotics. However the isolates were sensitive to Ciprofloxacin, Danofloxacin and Norfloxacin (100%) followed by Cephridine and Sulfamethxazole-trimethoprim (73.8% for each).

Similar results were obtained by Lee *et al.,* (2003) and Gorman and Adley (2004). Also, resistance to Tetracycline, Amoxycillin and

Ampicillin (92.9%, 88.1% and 78.6%, respectively) were detected which agreed with Botteldoorn *et al.,* (2004) and Johnson *et al.,* (2005). It was reported that multi-drug resistant Salmonella serovars cause severe and septicemic salmonellosis more frequently than those which are non-resistant (Helms *et al.,* 2002 and Gupta *et al.,* 2003).

The high incidence of antibiotic resistance among the tested salmonellae may be due to the misuse of antibiotics in addition to under-dosing and using the antibiotics as feed additives.

The pathoginicity testing of the isolated serovars in one-day old chicks (Table 4) revealed a variation in the degree of virulence in correlation to the variation of the type of serovar and route of infection. The mortality rates ranged from 70% -100% in case of oral infection, while reached 100% mortalities in all groups of chicks infected intraperitoneally. However, many factors can influence the relative pathogenicity of Salmonella in chicks, which includes the age of the chickens at the time of infection, route of infection, presence of competing bacteria in the intestinal tract and the dose of infection (Cox *et al.,* 1990; Cooper *et al.,* 1994 and Bailey *et al.,* 2001).

Mortalities appear within 24 hours after intraperitoneal infection and within 48-72 hours after oral infection. The main clinical symptoms were pyrexia, diarrheoa and inability to stand while the postmortem lesions revealed congestion of all internal organs specially in intraperitoneal infection associated with the isolation of the inoculated serovar. These results agreed with Bailey *et al.,* (2005) who recovered Salmonella from liver, thymus, spleen, bursa and ceca within 24 hours after oral inoculation.

The outer membrane proteins (OMPs) of salmonella and its compositions have been a subject of growing interest during the last few decades (Roushdy, 1998). In this work, (OMPs) of the isolated Salmonella serovars have been analyzed and electrophoresis' profiles have been determined by sodium dodocyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and resolved into protein bands as shown in Photo (1) and Table (5)

SDS-PAGE results revealed the determination of 6-10 protein bands ranging from 13.631 -200.5 kilo Dalton (KD). Nearly similar results were obtained by El-Reedy *et al.* (2007) who identified about 12 different protein bands ranging from 22-289 KD by SDS-PAGE analysis of 12 different salmonella serotypes isolated from poultry. Also there were common protein bands at 15, 26 and 35 KD identified in all isolated Salmonella serovars which agreed with the results obtained by

Ames (1973), Sarasombath *et al.* (1988) and Joradat and Zawistowski (1998) who detected the presence of 35 KD protein band in all examined salmonellae. On the other hand, *S. cerro* was characterized by 3 deeply stained protein bands at 120, 60.15 and 26 KD.; *S. enteritidis* was characterized by 4 deeply stained protein bands at 150.5, 100.66, 75.1 and 620.5 KD; *S. infantis* has 3 deeply stained protein bands at 180.2, 75 and 62.112 KD; *S. montivideo* characterized by 2 deeply stained protein bands at 150.9 KD and 100 KD, while *S. typhimurium* obtained 5 deeply stained protein bands at150, 100.25, 75.4, 62.71 and 36.532 KD. It is clear that the protein composition in relation to Salmonella serovars which agreed with the results obtained by Roushdy (1998).

SDS-PAGE immuonoblot procedure provided a rapid method for providing serological evidence of infection with Salmonella (Chart *et al.*, 1997). In this study, SDS-PAGE immuonoblotting detected 10-13 antigenic protein bands ranged from 11.8-147.3 KD as shown in Photo (2) and Table (6) with the detection of more protein bands which could not be detected in gel electrophoresis. This may be due to that immunoblot is more specific technique than gel electrophoresis so any epitopes can be captured by antibodies. It was noticed that the highest antigenicity protein band of *S. typhimurium* was detected at 35.4 KD, while in *S. enteritidis* was detected at 29 KD; in *S. montivideo* at 39.8 KD; in *S. infantis* at 25.128 and 34.091 KD while *S. cerro* had the highest antigenic protein band at 143.08. Similar results obtained by Nese *et al.* (2003) who found that *Salmonella typhimurium* isolates contain OMPs have the highest antigenicity common fractions at 36-43 KD. Moreover, SDS-IMMUNOBLOT of the five Salmonella serovars showed the presence of common protein bands at 15, 29 and 35 KD which may constitute the common genus antigen. These results agreed with that obtained by Nasef (1995) who studied the immunogenicity of Salmonella common protein detected at 29 KD and revealed the immunologic specificity of this band against antisera of different studied salmonella serotypes. Also Fathi (2004) recognized the 35 KD protein band in all examined salmonellae.

On the other hand, it was noticed the presence of shared immunogenic protein bands between different Salmonella serovars which may be the reason of cross reaction between Salmonella serovars. Similar results were obtained by Timoney *et al.* (1990) and Van Zijderveld *et al.* (1992) who observed the presence of cross reaction between different Salmonella serotypes specially group B and D1 in ELISA based on whole flagella antigen. Also, Cooper and Thorn (1996)

reported that rabbit sera raised against *Salmonella montivideo* reacted strongly with *Salmonella enteritidis* flagellins. The cross reactions may be attributed to common epitopes present on different flagellins.

In conclusion, this study has been able to identify the differences in the organization of the proteins of the isolated Salmonella serovars which demonstrated the potential use of SDS-PAGE analysis and immuonoblotting as a recent and accurate techniques for detection of salmonellosis in chickens and the use of immunogenicity of different detected immunogenic bands to serve as components of an effective subcellular vaccine for poultry salmonellosis.

REFERENCES

Abd-Allah, M. (1995): Microbiological studies of naturally and experimentally occurring Salmonella organisms in poultry and their environments in El- Fayoum governorate. PH.D thesis, (Bacteriology) Fac. Vet. Med., Cairo Univ., (Beni-Suef branch).

Abd-El Hamid, H.S.; Torkey, H.A.; AL Shaboury, F.A.; Meran, M.A. ; Sleim Ellakany and Awad, A.M. (2004): Epidemiological studies on salmonellosis in poultry. The 4th Scientific conference, 2-4 October 2004, Fac. Vet. Med., Alexandria University.

Ahmed, M.; Hameed, A. and Sultana, K. (1998): Antimicrobial susceptibility and whole cell protein analysis of Salmonella isolates from poultry. Pakistan J. Zool., 30(4): 365-369.

Ames, G.F. (1973): Antigenically stable 35 KD outer membrane protein of Salmonella. Food and Agricultural Immuonology, 10 (3): 259-270.

Bailey, J.S.; Stern, N.J.; Fedorka-Cray, P.; Craven, S.E.; Cox, N.A.; Cosby, D.E.; Ladely, S. and Musgrove, M.T. (2001): Sources and movement of Salmonella through integrated poultry operations: A multistate epidemiological investigation. J. Food Prot. 64: 1690-1697.

Bailey, J. S.; Cox, N.A.; Cosby, D.E. and Richardson, L.J. (2005): movement and persistence of Salmonella in broiler chickens followed oral and intracloacal inoculation. J. Food Prot., 68(12): 2698-2701.

Bakshi, C.S.; Singh, V.P.; Malik, M.; Singh, R.K and Sharma, B. (2003): 55kb plasmid and virulence – Associated genes are positively correlated with *Salmonella enteritidis* pathogenicity in mice and chickens. Vet., Res. Common. 27: 425 – 432.

Barnhart, H.M.; Dreesen, D.W.; Bostien, R. and Pancorbo, A.C. (1992): Prevalence of J. Food Prot and other serovars on ovary of layer hens at slaughter time. J. Food Prot., 54 (7): 488-491.

Berkhoff, H.A. and Vinal, A.C. (1986): Congo red medium to distinguish between invasive and non-invasive *Echerichia coli* pathogenic for poultry. Avian Dis., 30(1): 117-121.

Botteldoorn, N.; Herman, L.; Rijpens, N. and Heyndrick, M. (2004): Phenotypic and molecular typing of Salmonella strains revealed different contamination sources in two commercial pig slaughter houses. Appl, Environ. Microbiol., 70 (9): 5305- 5314.

Chart, H.; Rowe, B. and Cheesbrough, J.S. (1997): Serological response of patients infected with Salmonella typhi. J. Clin. Pathol., Nov. 50 (11): 944-946.

Collier, L.; Balows, A. and Sussman, M. (1998): Microbiology and microbial infection. Topley and Wilson's 9th Ed.

Cooper, G.L. and Thorns, C.J. (1996): Evaluation of SEF14 fimbrial dot blot and flagellar western blot tests as indicators of Salmonella enteritidis infection in chickens. Vet. Rec., 138:149-153.

Cooper, G.L.; Venables, L.M.; Woodward, M.J. and Hormaeche, C.E. (1994): Invasiveness and persistence of *Salmonella enteritidis*, Salmonella typhimurium and genetically defined *Salmonella enteritidis* aroA strain in young chickens. Infect. Immun., 62 (11): 4739- 4746.

Cox, N.A.; Bailey, J.S.; Blankenship, L.C.; Meinermann, R.J.; Stern, N.J. and McHan, F. (1990): fifty percent colonization dose for *Salmonella typhimurium* administered orally and intracloacally to young broiler chicks. Poultry Sci., 69: 1809-1812.

Dorn, C.R.; Silapanuntakul, R.; Angrick, E.J. and Shipman, L.D. (1992): Plasmid analysis and epidemiology of *Salmonella enteritidis* infection in three commercial layer flocks. Avian Dis., 36: 844-851.

El-Reedy, S.A.; Hussein, K. Eldeen and Jihan M. Badr (2007): Protein analysis for comparison of Salmonellae isolated from different species of poultry. Beni- Suef Vet. Med. J., july, 17 (2): 1-9.

Fadl, A.A.; Venkilanarayanan, K.S. and Khan, M.I. (2002): Identification of *Salmonella enteritidis* outer membrane proteins expressed during attachment to human intestinal epithelial cells. J. Appl. Microbiol., 29(1):180-186.

Fathi, A.A. (2004): Protective immunity induced by outer membrane proteins and lipopolysaccharides against Salmonella infection. 6th scientific conference of the Egyptian Veterinary poultry Association, Sept., 25th-27th (2004), pp, 232-247.

Gorman, R. and Adley, C.C. (2004): Characterization of *Salmonella enterica* serotype Typhimurium isolates from human, food and animal sources in the republic of Ireland. J. Clin.Microbiol., 42(5): 2314-2316.

Gupta, A.; Fontana, J.; Crowe, C.; Bolstorff, B.; Stout, A.; Van Duyne, S.; Hoekstra, M.P.; Whichard, J.M.; Barrett, T.J. and Angulo, F.J. (2003): Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded - spectrum cephalosporins in United States. J. Infect. Dis., 188: 1707-1716.

Hassan, W.M.M.; Oraby, F.A.I. and Hassan, A.M. (2003): Comparative studies ondifferent isolates of avian salmonellae by Sodium Dodocyl Sulphate Polyacrylamide gel electerophoresis (SDS-PAGE). J. Egypt. Vet. Med. Assoc., 63(2): 65-72.

Helms, M.; Vastrup, P.; Gerner-Smidt, P. and Mobak, K. (2002): Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium.* Emerg. Infect. Dis., 8: 490-495.

Hofer, E.; Silra-Filho, S.J.; Maura, E. and Reis, F. (1998): Salmonella serovars isolated from feedstuff and poultry feeds in Brazil. Pesquisa Veterinaria Brasileira, 18(1): 21-27.

Holt, J.G.; Krieg, N.R.; Sneath, P.A.H.; Staley, J.T. and Williams, S.T. (1996): Bergey´s manual of determinative bacteriology, 9th ed., Williams and Wilkins, Baltimore, Maryland, USA.

Johnson, J.M.; Rajic, A. and McMullen, L.M. (2005): Antimicrobial resistance of selected Salmonella isolates from food animals and food in Alberta. Can. Vet. J., 46 (2): 141-146.

Joradat, Z.W. and Zawistowski, J. (1998): Antigenically stable 35 KD outer membrane proteins of Salmonella. Food and Agricultural Immunology, 10(3): 259-270.

Kauffman, G. (1974): Kauffman White Scheme. WHO-BD172, h, Rev.1 Acta. Path. Microbiol. Sci. 61: 385.

Kim, C.J. and Nagaraja, K.V. (1991): An agar gel enzyme assay (AGEA) for simple detection of *Salmonella enteritidis* antibodies in chicken sera. Diag. Microbiol. Infect. Dis., 14: 203-208.

Laemmeli, U.K. (1970): Cleavage of structural protein during the assembly of head of bacteriophage T4. Nature, 224: 680-684.

Le Minor, L.; Veron, M. and Popoff, M.Y. (1982): Proposal of Salmonella nomenclature. Annales de Microbiologie, 133B, 245-254.

Lee, Y.J.; Kim, K.S. and Kwon, Y.K. (2003): Biochemical characteristics and antimicrobial susceptibility of *Salmonella gallinarum* isolated in Korea. J. Vet. Sci., 4 (2): 161-166.

Mallinson, E.T. and Snoeyenbos , G. H. (1989): Salmonellosis: Cited in: A laboratory manual for the isolation and identification of avian pathogens . pp. 3 – 11, 3 rd. Ed., by Purchase, H.G.; Chairman, L.H.Arp; Domermuth, C.H. and Pearson, J.E.. Copies from: American Association of Avian Pathologists.

Murugkar, H.V.; Rahman, H.; Ashkor Komar and Bhattacharyya, D. (2005): Isolation, phage typing and antibiogram of Salmonella from man and animals in northern India. Indian J. Med. Res., (122): 237-242.

Nasef, S.A.A. (1995): Refinement of common antigen for detection of fowl paratyphoid carriers. Ph. D. Thesis (Poultry Diseases), Fac. Vet. Med., Cairo Univ.

National Committee for Clinical Laboratory Standerds (NCCLS) (2003): Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved StandardM31-A2. Villanova, PA, USA.

Nese, A.; Osmen, S.; Ali, K. and Kurtulus, T. (2003): Immunogenecity and specificity of Salmonella typhimurium outer membrane antigens. J. Cell and Molecular Biology, 2: 91-97.

Novac, S. and Polaharova, A. (1993): Occurrence of *Salmonella enteritidis* in poultry in various districts of Slovakia in 1990-1992. Veterinarsivi, 43(5): 193-194.

Ochea-Reparaz, J.; Sesma, B.; Alvarez, M.; Jesus, M.; Juan, M. and Gamazo, C. (2004): Humoral immune response in hens naturally infected with *Salmonella enteritidis* against outer membrane proteins and other surface structural antigens. Vet. Res., 35: 291-298.

Poppe, C. (2000): Salmonella infections in the domestic fowl. In: Salmonella in domestic animals. Ed. By Wray, C. and Wray, A. CABI publishing, CAB international, Wallingford, UK.. pp. 107-132

Qadri, F.; Hossain, S.A.; Giznar, I.; Haidar, K. ; Ljungh, A.; Wadstrom, T. and Sock, D.A. (1988): Congo red binding and salt aggregation as indicators of virulence of *Shigella species* . J. Clin. Microbiol., 26: 1343-1348.

Roushdy, S.M. (1998): Salmonella outer membrane proteins – immunogenic properties in mice. Ph.D. thesis, Fac., Vet., Med., Cairo Univ.

Sarasombath, S.; Lertmemongkolchai, G. and Banchiun, N. (1988): Characterization of monoclonal antibodies to protein antigen of Salmonella typhi. J. Clin. Microbiol., 26(3): 508- 512.

Shahata, M.A. (1995): Some studies on paratyphoid infections in poultry in upper Egypt. Ph.D. thesis (Poultry Diseases), Fac. Vet. Med., Assiut Univ.

Talbot, P.J.; Knobler, R.L. and Buchmeier, M.J. (1984): Western and dot immunoblotting analysis of viral antigens and antibodies: application of murine hepatitis virus. J. Immunol. Methods, 73: 177-188.

Timoney, J.F.; Sikora, N.; Shivaprasad, H.L. and Opitz, M. (1990): Detection of antibody to Salmonella enteritidis by gm flagellin-based ELISA. Vet. Rec., 127:168-169.

Van Zijderveld, F.G.; Van Zijderveld, Van Bemmel, A.M. and Ankotta, J. (1992): Comparison of four different enzyme – linked immunosorbant assays for serological diagnosis of Salmonella enteritidis infections in experimentally infected chickens. J. Clin. Microbiol., 30: 2560-2566.