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Protein analysis for comparison between Salmonellae isolated from different poultry species

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A total of 620 egg samples from different species (chickens, ducks and ostriches) and 1615 poultry samples (chickens, ducks, pigeons, quails, turkeys and ostriches) were examined for salmonella infection. 12 salmonella isolates were obtained from the egg samples (1.9%) and 67 isolates from poultry samples (4.1%). Salmonella isolates were serotyped into *S. enteritidis* (25 isolates), *S. typhimurium* (17 isolates), *S. infantis* (12 isolates), *S. montivideo* (7 isolates), 3 isolates for each of *S. rubislaw* and *S. cerro* , 2 isolates for each of *S. virginia*, *S. agona*, *S. poona*, and *S. derby and* 1 isolate for each of *S. sandiago* and *S. kentucky*. The incidence of isolation from different poultry species was discussed in details. Antibiogram of the isolated salmonellae against 10 different antibiotics revealed that norofloxacin, ciprofloxacin, cepheridin and gentamycin gave the highest activity against different salmonella isolates while amoxicillin, tetracycline, and nitrofurantoin showed the highest resistance rate. Pathogenicity of the isolated serovars was tested in chickens. All isolates were found pathogenic with various degree of virulence. SDS-PAGE protein analysis for the salmonella isolated form different poultry species revealed 12 protein bands ranged from 22-289 kDa. The differences were insufficient for reliable differentiation between the isolates and accordingly, it could be used beside other molecular techniques in differentiation between the salmonella strains.

Salmonellae are widespread in the environment worldwide resulting in human and animal diseases and costing many billions of pounds each year (Morales and Thuman, 1993). Among all animal species salmonella are most frequently reported from poultry and poultry products (Gupta and Verma, 1993; Rahman *et al*., 1997; Abd EL-Hamid *et al*., 2004 and Murugkar *et al*., 2005). Poultry products are known to be significant reservoir for salmonella and the most important source of *Salmonella enteritidis* infection in humans. On the other hand, with great expansion of the poultry industry, the wide spread occurrence of avian salmonellosis has ranked it as one of the most important egg-borne bacterial diseases of poultry (Hayes *et al*., 1999; Davis and Berslin, 2001; Molbak and Neimann, 2002). Because of the large population at risk there is an increase active nationwide programmes for their isolation and identification (Waltman and Malison, 1995; Waltman; 2000; Rybott *et al*., 2004)

In recent years, antibiotic resistance in salmonellae has assumed alarming proportions worldwide (Cruchaga *et al*., 2001; Murughar *et al*., 2005).

Monitoring drug resistant pattern among salmonella isolates is vital to the clinician in regard to treatment of the diseased cases and to produce an important tool for devising a comprehensive chemoprophylactic and chemo-therapeutic drug, schedule on the flock basis within geographical areas. The food and drug administration centers for disease control and prevention and others believe that agricultural misuse of antibiotics accounts for the majority of increases in antibiotic resistant isolates (Tollefson *et al*., 1999) and that many lead to public health threat (Witte, 1998).

Analysis of the whole–cell protein patterns has been used extensively to the study of the differences among bacterial genera, species and strains (Walia *et al*., 1988). Outer membrane protein analysis has proved to be useful technique in the characterization of *Salmonella* (Fadl *et al*., 2002; Ochea-Reparaz *et al*., 2004).

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The goal of this study was to elucidate the following points; i) Incidence of salmonellae in different poultry species with spotlighting the most prevalent isolated Salmonella serovars; ii) Determination of the antibiogram of different salmonella isolates; iii) Detection of the pathogenicity of different isolated serovars in day-old chicks; iv) Studying the whole–cell protein analysis of these isolates by SDS-PAGE.

Materials and Methods

Samples. A total of 620 eggs (including 450 chicken eggs, 150 duck eggs and 20 Ostrich eggs) either fertile or infertile were obtained from different farms from January 2004 to December 2005. A total of 1615 samples collected from liver, spleen, intestine (ceacum and cecal tonsils), bone marrow, yolk sac and heart blood of living or freshly dead cases of different poultry species (820 chickens, 230 ducks, 225 pigeons, 175 quails, 130 turkeys and 35 Ostriches). The collected samples were subjected to bacteriological examination.

Isolation of *Salmonellae*. The isolation of *Salmonellae* from different samples was carried out according to (Waltman *et al*., 1998)

Identification of the isolates. Serological identification was done using Kauffman white scheme described by Edwards and Ewing, (1986) and Kauffmann, (1974) by using polyvalent and monovalent (O) and (H) antisera.

Antibiotic sensitivity test. In vitro susceptibility testing of different salmonella isolates was determined against different commercial antibiotic discs using disc diffusion method described by (Quinn *et al*., 1994).

Pathogenicity test. One hundred and fourty one-day-old chicks were used in this test. They were divided into 14 groups (10 birds/group). The first 13 groups were used for pathogenicity testing of different salmonella isolates, while the last one was used as non-infected control group. The test was done according to (Bakshi *et al*., 2003). Postmortem examination and reisolation of the infected microorganisms from internal organs were also, carried out.

Sodium Dodecyl sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE). Total protein profile of the salmonella isolates was carried out using sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by the method described by Laemmli (1970) using miniprotein electrophoresis system (BioRad).The samples were electrophoresed for 1 h at 100V. Each run included prestaine protein marker (Invitrogen Co.). The gels were stained with Commassie brilliant blue and destained using distaining solution according to (Bushuk *et al*., 1999; Demiralp *et al*., 2000).

Total protein analysis. The protein analysis of the SDS-PAGE for the salmonella isolates was done using the computer analysis program (Gel-Pro Analyzer V 3.1).

Results and Discussion

Bacteriological examination of the egg samples from different poultry species revealed an overall incidence of 1.9 % of the examined samples [620], (Table 1). These results are greatly in agree with those obtained by Moustafa (1982) who recorded an incidence of 1.7 % of salmonella isolation from infertile egg samples. The lowest incidence of isolation was obtained with chicken egg samples (1.6 %) that agree with the results obtained by EL-Agroudi and Awad (1966) who recorded only 88 salmonella positive eggs out of 5000 examined hen eggs which constituted an incidence of 1.7 %.On the other hand, incidence of salmonella isolation from duck eggs was the highest (3.3 %) and mostly identified *as S. typhimurium* (Table 2, 3). It was reported that in spite of the very thick cuticle covering the duck eggs, but it was observed that it may be removed as a result of being scraped by the parent or another duck while the cuticle is still soft after oviposition or due to the habit of the ducks to defecate in the nesting boxes that contaminate egg shell and increase the probability of the infection. There was an association between consumption of duck eggs and gastrointestinal illness which was largely proved to be due to the presence *Salmonella typhimurium* (Henry, 2000).

It was found that the most prevalent salmonella serovars isolated from eggs of different poultry species was *S. enteritidis* [41.7 5%] (Table 2,3). Cases of salmonellosis caused by *S. enteritidis* had dramatically increased from 5 % to 26% during the period 1976 to 1994. (U.S. Department of Agriculture 1995) and the major source of these infections was properly grade A table eggs (Shah *et. al*. 1991). Many studies have established that *S. enteritidis* contaminates eggs when the organism is passed from the infected reproductive tissue of the hens rather than the shell, to the contents of contaminated eggs (Shivaprasad *et al*. 1990; Humphrey, 1994).

On the other hand, it was found that the highest incidence of salmonella isolation was obtained from ducks [7.8 %] (Table 4) which were mostly belonged to *S. enteritidis* (6

Table (1): Incidence of salmonella isolation from egg samples from different poultry species.

Examined eggs		Salmonella positive eggs	
Species	**No.**	**No.**	**Percent**
Chicken eggs	450	7	1.6
Duck eggs	120	4	3.3
Ostriches eggs	50	1	2.0
Total	620	12	1.9

Table (2): Salmonella serovars isolated from eggs from different poultry species.

Salmonella serovars	Chicken eggs	Duck eggs	Ostriches eggs	Total	
				No.	**Percent**
S. enteritidis	3	1	1	5	41.7
S. typhimurium	1	2	0	3	25.0
S. infantis	2	0	0	2	16.7
S. montivideo	1	0	0	1	8.3
S. virginia	0	1	0	1	8.3
Total	7	4	1	12	100

isolates) followed by *S. typhimurium* (5 isolates). These results agreed with Hui and Das (2001) who isolated 15 salmonella strains (5.36 %) out of 280 samples from dead, diseased and apparently healthy ducks at different farms in Bengal, India and serotyping of the isolated strains revealed that they were grouped into 2 different serogroups *S. enteritidis* and *S. typhimurium* constituting 80 % and 20 % respectively.

Other salmonella serovars isolated from ducks includes *S. infantis* (3 isolates). *S. montivideo* (2 isolates), *S. agona* and *S. virginia* (one isolate from each). The same serovars were isolated from ducks by other authors (Simko, 1988; Maff, 1997).

The incidence of salmonella isolation from chicken samples was 4.6 % (Table 4) , including *S. enteritidis* (43.2 %), *S. typhimurium* (18.4 %), *S. infantis* (15.8 %), *S. montivideo* (10.5 %) , *S. cerro* (7.9 %), *S. rubislaw* (5.3 %), *S.derby*, *S. poona* and *S. Kentucky* (2.6% for each) (Table 5).

These results are in agreement with that obtained by Hassan *et al*. (2003) who isolated 35 salmonella isolates (5.51 %) out of 635 examined chicken samples at different growth stages and were identified serologically to *S. typhimurium*, *S. enteritidis*, *S. pullorum* and *S. rubislaw*. Abd-EL-Hamid *et al*. (2004) isolated *S. enteritidis*, *S. typhimurium*, *S. kentucky*, *S. montivideo* from duck and chicken samples of different growth stages, while Murugkar *et al*. (2005) isolated 231 cloacal swabs from diarrhoeic birds and the isolates were serotyped *S. typhimurium* (12 isolates), *S. gallinarum* (12 isolates), *S. enteritidis* (8 isolates) and *S. pratyphi* B (2 isolates). These results disagree with Mojnaric *et al*. (2003) who detected lower incidence of salmonella isolation (2.75%) from chicken samples during the year 2002 in north-western Croatia, mostly confirmed to be *S. enteritidis* (82%) and other salmonellae (18%).

Turkeys isolates constituted 7.4% of the total salmonella isolates from different poultry species (Table 5). The isolated strains were serotyped as *S. enteritidis*, *S. typhimurium*, *S. rubislaw*, *S. derby* and *S. sandiago*. The same serotypes were isolated previously by several authors (Pomeroy *et al*., 1984; Hirschman and Seidel, 1992; Hafez *et al*., 1997). Salmonellosis in turkeys is distributed world wide and resulted in severe economic losses which caused by high poult mortality during the first 4 weeks of age, high medication costs, reduction in egg production in breeder flocks, poor poult quality and high costs for eradication and control measures.

Pigeons and quails had the least incidence of salmonella isolation among different species of poultry, 1.3% and 1.2% respectively (Table 4). *S. typhimurium* was the most prevalent isolated serovars from quails (2 isolates), followed by *S. agona* (1 isolate) (Table 5), while the isolates from pigeons belonged to *S. typhimurium* and *S. infantis*. These results agree with Cizek *et al*.

Table (3): Antigenic structure of different salmonellae isolated from poultry.

Salmonella serovars	Sero-group	Antigenic structure		
		[O]	[H]	
			Phase (1)	Phase (2)
S. agona	B	1,4,12	f,g,s	-
S. derby	B	1,4,[5],12	f,g	[1,2]
S. sandiego	B	4,[5],12	e,h	e,h,z_{15}
S. typhimurium	B	1,4,[5],12	i	1,2
S. infantis	C1	6,7	r	1,5
S. Montevideo	C1	6,7	g,m,s,[p]	-
S. kentucky	C3	8,20	i	z_6
S. virginia	C3	8	d	1,2
S. enteritidis	D1	1,9,12	g,m	-
S. rubislaw	F	11	r	e,n,x
S. poona	G1	1,13,22	z	1,6
S. cerro	K	8,14,18	z_4,z_{12}	

Table (4): Incidence of salmonella isolation from different poultry species.

Poultry species	No. of examined cases	Salmonella positive cases	
		No.	Percent
Chicken	820	38	4.6
Ducks	230	18	7.8
Pigeon	155	2	1.3
Quails	245	3	1.2
Turkeys	130	5	3.8
Ostriches	35	1	2.9
Total	1615	67	4.1

(1994) who isolated salmonella from carrier pigeons in salmonella-free farms as well as Hudson *et al*. (2000) and Mosaad *et al*. (2000) who isolated *S. typhimurium* from wild and domesticated quails.

Concerning isolation of salmonella from Ostrich's samples only one case was salmonella positive (Table 4) and serotyped as *S. typhimurium*. This result is in agreement with that obtained by Gopo and Banda, (1997) who reported salmonella infection from Ostrich's samples. This result also agreed with Ley *et al*. (2001) who detected Salmonella and Campylobacter in examined Ostrich's carcasses.

Monitoring drug resistance pattern among different isolated salmonella serovars (Table 6) revealed that the highest number of isolates showed resistance against amoxicillin (37 isolates, 46.3%) and tetracycline (34 isolates, 42.5%) followed by nitrofurantoin (18 isolates, 22.5%), chloramphinicol (15 isolates, 18.8%), naldixic acid (11 isolates, 13.8%), sulphamethoxazon-trimethoprim (9 isolates, 11.3%) and gentamycin (7 isolates, 8.8%).The least resistance rates were detected against cepheridin, norofloxacin and ciprofloxacin (4 isolates for each, 5.0%). These results agree with HUI and Das, (2001) who found that most of salmonellae isolated from ducks were highly resistant to oxytetracycline followed by tetracycline and penicillin G. They also agree with Murugkar *et al*. (2005) who found that the most salmonella isolates were resistant to doxycycline (61.05%), ampicillin (51.57%), amoxicillin (45.26%), tetracycline (44.21%), nitrofurantoin (15.79%), trimethoprim (9.5%) and gentamycin (6.3%). They added that flouroquinolone group of antibiotics have the least rate among salmonella isolates.

Resistance to different antibiotics is of great concern since most of these antibiotics are added in the poultry feed as supplements and the obvious lack of control on the antibiotics usage may be the probable cause for their high resistance (Dorn *et al*., 1992).

The pathogenicity testing of the isolated serovars in day-old chicks (Table 7) revealed that *S. enteritidis*, *S. typhimurium*, *S. infantis* and

Table (5): Salmonella serovars isolated from different poultry species.

Salmonella serovars	Chicken		Ducks		Pigeon		Quails		Turkeys		Ostriches		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
S. enteritidis	13	34.2	6	33.3	0	-	0	-	1	20	0	0	20	29.9
S. typhimurium	7	18.4	5	27.8	1	50	2	66.6	1	20	1	1.5	17	25.4
S. infantis	6	15.8	3	16.7	1	50	0	-	0	-	0	0	10	14.9
S. montevideo	4	10.5	2	11.1	0	0	0	-	0	-	0	0	6	9
S. rubislaw	2	5.3	0	0	0	0	0	-	1	20	0	0	3	4.5
S. cerro	3	7.9	0	0	0	0	0	-	0	0	0	0	3	4.5
S. derby	1	2.6	0	0	0	0	0	-	1	20	0	0	2	3.0
S. poona	1	2.6	0	0	0	0	0	-	0	0	0	0	2	3.0
S. sandiago	0	-	0	0	0	0	0	-	1	20	0	0	1	1.5
S. Kentucky	1	2.6	0	0	0	0	0	-	0	0	0	0	1	1.5
S. agona	0	-	1	5.6	0	0	1	33.3	0	0	0	0	2	3.0
S. virginia	0	-	1	5.6	0	0	0	-	0	0	0	0	1	1.5
Total	38	56.7	18	26.9	2	3	3	4.5	5	7.5	1	1.5	67	100

Table (6): Antibiotic Resistance pattern of salmonellae isolated from poultry

Salmonella Serotype	Source		No. of salmonella isolates resistant to antibiotics										
	Origin	No.	Total	F	NA	C	AML	GM	TE	CE	SXT	CIP	Nor
S. enteritidis	Eggs poultry	5 20	25	6	5	6	15	2	10	1	6	2	2
S. typhimurium	Eggs poultry	3 17	20	5	3	4	8	1	8	1	1	0	0
S. infantis	Eggs poultry	2 10	12	4	2	2	5	1	5	1	0	0	1
S. montevideo	Eggs poultry	1 6	7	2	1	0	2	1	4	0	0	1	0
S. rubislaw	poultry	3	3	0	0	1	2	0	1	0	1	0	1
S. cerro	poultry	3	3	1	0	1	1	0	2	0	0	0	0
S. virginia	Eggs poultry	1 1	2	0	0	0	0	0	1	0	0	1	0
S. agona	poultry	2	2	0	0	1	1	0	0	0	1	0	0
S. poona	poultry	2	2	0	0	0	1	1	1	0	0	0	0
S. derby	poultry	2	2	0	0	0	0	0	1	1	0	0	0
S. sandiago	poultry	1	1	0	0	0	1	1	0	0	0	0	0
S. kentucky	poultry	1	1	0	0	0	1	0	1	0	0	0	0
Total	-	80	80	18	11	15	37	7	34	4	9	4	4

F = Nitrofurantoin **AML** = Amoxicillin **CE** = Cephridin
NA = Naldixic acid **GM** = Gentamycin **CIP** = Ciprofloxacin
C = Chloramphinicol **TE** = Tetracycline **Nor** = Norfloxacin
SXT=sulphamethoxazone-trimethoprim

S. derby were highly pathogenic (100% mortality) followed by, *S. cerro*, *S. agona* and *S. virginia* (95% mortality) while *S. rubislaw*, *S. kentucky*, *S. montivideo*, *S. santiago* and *S. poona* were less pathogenic (90%, 85%, 80%, 80%, and 80% respectively). Most of the mortalities occur within 24-48 h post-infection accompanied with reisolation of the injected serovars from different internal organs. The dead birds showed signs of septicemia include typical congestion of blood vessels and internal organs with mottling and enlargement of the liver and spleen. A similar picture was recorded by Synoeynbos *et al*. (1986) and Lee, (1987).

The variation in the degree of virulence could be attributed to the type of serovars and also the routes of infection as the chicks are more susceptible to salmonella infection by inhalation

Table (7): Pathogenicity of salmonella serovars isolated from poultry

Salmonella Serovars	No. of infected chicks	Route of infection	Dose of infection CFU	Mortality		Salmonella re-isolation				
				No.	%	Heart	Liver	Bone marrow	Lungs	kidney
Control -ve	20		0	0	0	0	0	0	0	0
S. enteritidis	20			20	100	20/20	20/20	15/20	18/20	16/20
S. typhimurium	20			20	100	20/20	20/20	18/20	15/20	16/20
S. infantis	20			20	100	20/20	20/20	18/20	19/20	15/20
S. derby	20			20	100	20/20	20/20	18/20	19/20	15/20
S. cerro	20			19	95	18/19	19/19	17/19	15/19	13/19
S. virginia	20	I / P	$3X10^8$ CFU	19	95	19/19	19/19	17/19	19/19	17/19
S. agona	20			19	95	18/19	19/19	16/19	17/19	15/19
S. rubislaw	20			18	90	18/18	18/18	16/18	15/18	14/18
S. kentucky	20			17	85	14/17	17/17	14/17	16/17	15/17
S. montevideo	20			16	80	16/16	16/16	13/16	15/16	10/16
S. poona	20			16	80	13/16	16/16	11/16	12/16	11/16
S. sandiago	20			16	80	15/16	16/16	10/16	14/16	13/16

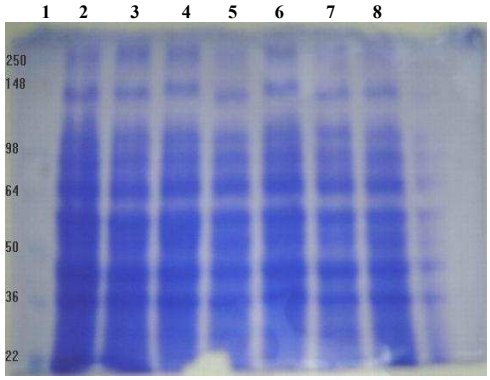

Fig (1): SDS-PAGE protein profile of salmonella isolated from different species of poultry and eggs. Lane (1): protein marker, Lane (2):S.rubislaw, Lane (3):S.sandiago, Lane (4):S.derby, Lane (5):S.typhimurium, Lane (6):S.agona, Lane (7):S.virginia, Lane (8):S.enteritidis.

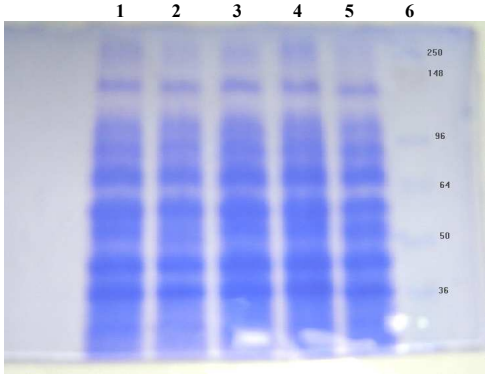

Fig (2): SDS-PAGE protein profile of salmonella isolated from chicken and chicken eggs. Lane (1): S.montevideo, Lane (2):S.kentucky, Lane (3):S.infantis, Lane (4):S.cerro, Lane (5):S.poona, Lane (6): protein marker.

and parentral routes than via oral route (Baskerville *et al*., 1992; Poppe *et al*., 1993; Cooper *et a*l., 1994).

SDS-PAGE protein analysis of the isolated salmonella from different poultry revealed about 12 protein bands (Tables 8,9, Fig 1,2). The protein bands ranged from 22-289 kDa. The differences were insufficient for reliable differentiation between the isolates. The 29 kDa protein band was the common antigen which represent SCOPA as mentioned by Soad,(1995), while there were protein bands 67, 57, 43, 36, 33, 22 kDa in all the isolates which agree with the results of Ochea-Reparaz *et al*. (2004) who mentioned that Flagellin 53 and 45.1 kDa, Porins 35-36 kDa Omp A 34 kDa and Omp 22.1 kDa, Nese *et al*. (2003) who found that the *S. typhimurium* isolates contained OMPs with the molecular sizes 70 kDa and the highest antigenicity common protein fraction was 36-43 kDa. and Helmuth *et al*. (1985) who mentioned that the *S. typhimurium* strains generally contained OMPs of 37, 40 and 41.7 kDa. The protein band of 119.6 was only in *S. virginia* and *S. typhimurium*.

In our results, the differences were insufficient for reliable differentiation and the protein analysis may be used beside other molecular techniques to differentiate among the salmonella strains.

Table (8): SDS-PAGE whole cell protein analysis of salmonella isolated from different species of poultry and eggs.

Protein Marker	Lane 2 S.rubislaw (Turkey)	Lane 3 S.sandiago (Turkey)	Lane 4 S.derby (Turkey)	Lane 5 S.typhimurium (Ostrich)	Lane 6 S.agona (Quail)	Lane 7 S.virginia (Duck's egg)	Lane 8 S.enteritidis (Duck's egg)
	289.1	289.1	289.1	289.1	289.1	289.1	289.1
250							
148							
	140.3	140.3	140.3		140.3		140.3
				119.6		119.6	
	105.4	105.4	105.4	105.4	105.4	105.4	105.4
98							
	87.8	87.8	87.8	87.8	87.8	87.8	87.8
	67.3	67.3	67.3	67.3	67.3	67.3	67.3
64							
	57.9	57.9	57.9	57.9	57.9	57.9	57.9
	51.9	51.9	51.9	51.9	51.9	51.9	51.9
50							
	43.3	43.3	43.3	43.3	43.3	43.3	43.3
	36.4	36.4	36.4	36.4	36.4	36.4	36.4
36							
	29.1	29.1	29.1	29.1	29.1	29.1	29.1
	24.1	24.1	24.1	24.1	24.1	24.1	24.1
22	22	22	22	22	22	22	22

Table (9): SDS-PAGE whole cell protein analysis of salmonella isolated from chicken and chicken eggs.

Lane 1 S.montevideo (chicken eggs	Lane 2 S.kentucky (chicken)	Lane 3 S.infantis (chicken)	Lane 4 S.cerro (chicken)	Lane 5 S.poona (chicken)	Protein Marker
289.1	289.1	289.1	289.1	289.1	250
					148
134.1	134.1	134.1	134.1	134.1	
					98
67.1	67.3	67.3	67.3	67.3	67.3
					64
57.9	57.9	57.9	57.9	57.9	
52.4	52.4	52.4	52.4	52.4	
					50
44.9	44.9	44.9	44.9	44.9	
36.2	36.2	36.2	36.2	36.2	36
33	33	33	33	33	
29	29	29	29	29	
24.1	24.1	24.1	24.1	24.1	
22	22	22	22	22	22

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تحليل البروتين للمقارنة بين عترات السالمونيلا المعزولة من الدواجن

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فى هذه الدراسة تم فحص ٦٢٠ عينة من بيض الدجاج و البط و النعام و ١٦١٥ عينة من الدجاج و البط و الحمام والسمان و الرومى و النعام لوجود عدوى السالمونيلا. و قد تم عزل ١٢ عترة من السالمونيلا من عينات البيض (١.٩%) و ٦٧ عترة سالمونيلا من الدجاج (٤.١ %). كما تم تصنيف عترات السالمونيلا إلى سالمونيلا إنتراتيدس (٢٥ عترة) ، سالمونيلا تيفيميوريوم (١٧ عترة) ، سالمونيلا إنفانتس (١٢ عترة) ، سالمونيلا مونتيفيديو (٧ عترات) ، سالمونيلا روبيسلو و سالمونيلا سيرو (٣ عترات من كل نوع) ، سالمونيلا فيرجينيا و سالمونيلا أجونا و سالمونيلا بونا و سالمونيلا ديربى (٢ عترة من كل نوع) و تم تصنيف سالمونيلا ساندييجو و سالمونيلا كينتاكى (عترة واحدة من كل نوع) و عن تأثير المضادات الحيوية المختلفة على عترات السالمونيلا المعزولة وجد أن النورفلوكساسين و السيبروفلوكساسين و السيفردين و الجينتاميسن قد أعطوا أعلى نتائج ضد العترات المختلفة من السالمونيلا المعزولة بينما أظهرت هذه العترات أعلى مقاومة ضد الأموكساسيلين و التيتراسيكلين و النيتروفيورانتوين ، كما تمت دراسة ضراوة هذه العترات فى الكتاكيت ووجد أنها جميعاً ضارية و لكن بدرجات متفاوتة و بتحليل بروتين خلايا عترات السالموبيلا المعزولة وجد أن التحليل الكهربائي بالبولى أكريلاميد جيل غير كافى للتفريق بين العترات و أنه لابد من إجراء اختبارات جزيئية أخرى لتعريف عترات السالمونيلا المختلفة.