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Monitoring *Salmonella* species contamination in imported horses and meat through Cairo International Airport

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Abstract

Quarantine against *Salmonella* through Cairo International Airport was assessed from July 2014 through July 2015 and from January 2014 through July 2015 for imported horses and meat; respectively. The samples included the fecal samples of imported horses (*n*=90), imported chilled meat (*n*=67) and imported frozen meat (*n*=63).Imported horses from Belgium, England, Germany, France, Emirates and Kuwait free from *Salmonella.* Although 1.5% of the imported chilled meat was positive for *Salmonella.* The most prevalent serotype isolated was *S. Kentucky*, imported from Australia in summer 2014, while 6.4% of the imported frozen meat was positive for *Salmonella*; the most prevalent serotype isolated were *S. Infantis, S. Hafnia, S.Typhimurim* and *S.Newport* imported in winter 2014, summer 2014 (South Africa), winter 2015 (Australia) and summer 2014 (U.S.A) respectively. Prevalence rate of *Salmonella* higher (60%) in summer season and decreased in winter (40%) but lower in autumn (20%), absence in spring. *S. Typhimurim* was sensitive to Florfenicol, Gentamycin and Streptomycin.

Key words : imported horse- Cairo Airport- imported meat-*Salmonella*.
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Introduction

Egypt requirements for imported animals and by products are governed by epidemiological situation of the country and by the law of Agriculture **No. 53, 1966 and Ministerial Decree No. 47, 1967** concerning veterinary quarantine, and its modifications.

Salmonella infection canaffect horses of all ages and range in severity from asymptomatic colonization to severe systemic illness.

S. Enteritidis and *S. Typhimurium* are the most commonly reported serovars in the European Union (EU), being associated with

52.3% and 23.3% of all confirmed human

infections (Salmonellosis), respectively. Since 2006, *S. Infantis* has been the third most common serovar in the EU due to ingestion of retail meat and meat products (**EFSA and ECDC, 2011).**

Salmonella species are considered to be among the most important food borne pathogens in the world and Salmonellosis is still one of the most widespread food borne bacterial illnesses in humans, with clinical manifestations ranging from asymptomatic state to severe disease **(Galanis *et al.* 2006)**

Material and Methods

1. Sampling from imported horses

A total of (90) samples were collected from healthy imported horses from Veterinary Quarantine of Cairo International Airport. All samples were collected from July 2014 through July 2015

2. Sampling from imported meat:

Samplings were performed following current governmental guideline (Ministry of industry and foreign trade). Samples from each imported consignment through Cairo International airport were collected and transferred to laboratories of General Organization for Export and Import Control, Ministry of Health and Animal Health Research Institute. A total (67) samples from imported chilled meat. A total (63) samples from imported frozen meat. All samples were kept in ice boxes and transferred without delay to the Laboratories. All samples were collected from January 2014 through July 2015.

3. Isolation and identification of *Salmonella*(ISO, 6579:2002)

Identification of *Salmonella* was performed according to ISO, 6579 (2002). From each sample, 25 g was placed in a sterile stomacher bag and 225 mL buffered peptone water. Samples were then homogenized using a stomacher for at least 2 minutes, followed by incubation at 37°C for 24 hours. Afterwards, 0.1 mL of pre-enriched broth was transferred into 10 mL of Rappaport-Vassiliadis medium (Oxoid, UK) and incubated at 41.5°C ± 0.5°C overnight, 24 hours. The enriched samples were then plated on toon Xylose Lysine

Desoxycholate (XLD) and Hektoen enteric agar (Sigma, USA) and incubated at 37°C for 24 hours.A typical *Salmonella* colony has a slightly transparent zone of reddish color and black center (XLD).A pink-red zone may be seen in the media surrounding the colonies. A typical *Salmonella* colony; black center with green zone (Hektoen)

4. Biochemical characterization of isolates

Biochemical characterization was based on standard techniques (***Quinn et al,* 2002**). All isolates that gave reactions typical of *Salmonella* in the tests and substrates were considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions are indole negative, negative urease, methyl red positive, Vogese Proskauer negative, citrate positive, produces H2S, lysine decarboxylase positive, oxidase negative.

5. *Salmonella* serotyping (OIE, 2004).

The first phase of serological typing was to serogroup. The isolates based on their somatic O-group antigens using commercially available polyvalent somatic antisera. Bacterial growth from an agar plate is emulsified in a small amount of physiological normal saline to form milky suspensions and one drop ofpolyvalent O-antisera was mixed with it on a slide or plate. The agglutination reaction was read within 60 sec.

Results

I. Results of isolated *Salmonella* from imported horses.

Table (1): Prevalence of *Salmonella* in imported horses.

Country	Breed	Type of samples	No of consignments	No of Samples	Purposes	No of samples / Seasons	+Ve/-Ve
Belgium	Warm blood	Fecal samples	9	40	Jumping	9 / summer 2014 12 /autumn 2014 19 / winter 2015	- ve
England	Warm blood	Fecal samples	4	16	Jumping	6 / summer 2014 7 / autumn 2014 3 / winter 2015	- ve
Germany	Warm blood	Fecal samples	3	13	Jumping	6 / autumn 2014 7 / winter 2015	- ve
France	Warm blood	Fecal samples	2	6	Racing	6/ winter 2015	-ve
Emirates	Arabian	Fecal samples	1	5	Racing	5 /spring 2015	-ve
Kuwait	Arabian	Fecal samples	2	10	Racing	10/summer 2015	-ve

II. Results of isolated *Salmonella* from imported meat.

Table (2): Prevalence of *Salmonella* in cuts of imported chilled meat

Country	Type of cuts	No of samples	*Salmonella*				Seasons	+Ve/ -Ve	Sero-typing
			+ve	%	-ve	%			
Australia	Beef cuts	23 samples	1	4.4 %	22	95.6%	6 winter 2014	- ve	-
							4 summer 2014	One positive *S.Kentucky*	Poly O8 H6
							4 autumn 2014	-ve	-
							6 winter 2015	- ve	-
							3 summer 2015	-ve	-
	Veal cut	5 samples					3 summer 2014	-ve	-
							2 autumn 2014	- ve	-

2. Frozen meat

Table (3): Prevalence of *Salmonella* in imported Beef Cuts of frozen meat from South Africa.

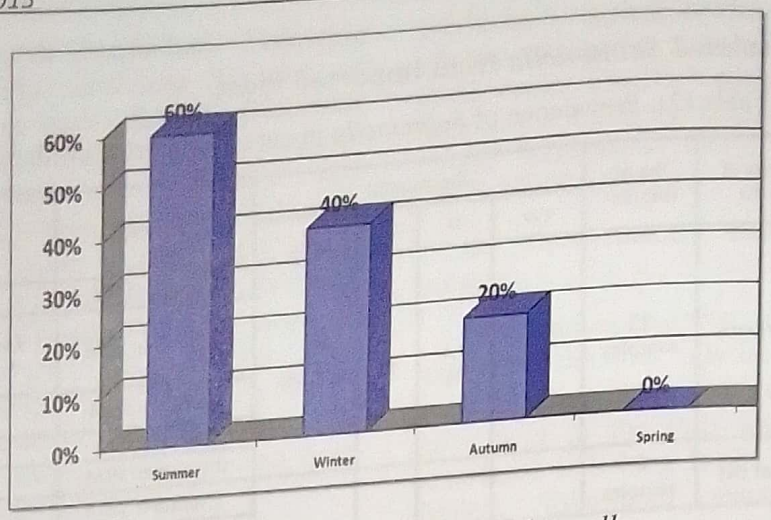
No of samples	*Salmonella*				Year	Seasons	+Ve/ -Ve	Serotyping
	+ve	%	-ve	%				
31 samples	2	6.5%	29	93.5%	2014	7 Winter	1 +ve *S.Infantis*	O 6,7,4 H 1,5
						3 Spring	- ve	-
						6 Summer	1+ve *S. Hafnia*	O 7,6 H —
						6 Autumn	- ve	-
					2015	7 Winter	- ve	-
						2 Summer	-ve	-

Table (4) Prevalence of *Salmonella* in imported Beef Cuts of frozen meat from Australia.

No of Samples	*Salmonella*				Year	Seasons	+Ve / - Ve	Serotyping
	+ve	%	-ve	%				
19 Samples	1	5.3%	18	94.7%	2014	5 Winter	-ve	-
						3 Spring		
						4 Summer		
						5 Autumn		
					2015	3 Winter	1+ve *S.Typhimurim*	Poly O 1,4 H 1,2

Table (5) Prevalence of *Salmonella* in imported Beef Cuts of frozen meat from USA.

No of samples	*Salmonella*				Year	Seasons	+Ve/ - Ve	Serotyping
	+ve	%	-ve	%				
13 Samples	1	7.7%	13	92.3%	2014	3 Winter	-ve	-
						4 Spring		
						2 Summer	1 +ve *S. Newport*	O 20,8,6 H h2 :1,2
					2015	3 winter	-ve	-
						1 Summer		



Fig(1): Seasonal incidence of *Salmonella*

Discussion

Horse Salmonellosis:

The data presented in table (1) revealed that examined imported horses through International Cairo Airport were free from *Salmonella*. The obtained results were disagreed with the results recoded by **AL Stad(1984), Ikeda and Hirsh (1985), Palmer *et al* (1985),), Traub-Dargatz*et al* (1990), Kim*et al* (2001), ward *et al* (2005 b) and Singh *et al* (2007).**The observed presence of *Salmonella* in other investigators due to isolated from diseased and hospitalized horses. It is obvious that absence of *Salmonella* isolation in the present study from imported horses through International Cairo Airport might be attributed to exported horses accompanied with veterinary health certificate and during the last 30 days prior of exportation they were isolated from any other horses with different health status and free from bacterial and viral diseases, they have been examined today and as far as can be determined show no clerical evidence of any epidemic, infections or contagious disease.

Chilled meat:

The prevalence of *Salmonella* in imported chilled meat(Table 2) was 1.5%. Table (2) showed that the most prevalent *Salmonella* serotype isolated from imported chilled meat was *Salmonella Kentucky* in summer season 2014 from Australia, while imported chilled meat from USA and South Africa was free from *Salmonella*. The obtained result were lower than that isolated by **Ahmed and Shimamoto (2013)** 1.9%, **Mayrhofer *et al* (2004)** 1.8% and **Tafida*et al* (2012)** 2.43%.According to quarantine regulations and general organization for Export & import in Egypt, imported meat must be free from *Salmonella*. The presence of low incidence of *Salmonella* imported from Australia (one consignment) can be attributed to cross contamination during handling at the slaughterhouse. Moreover, cross contamination during packaging and processing.

Frozen meat:

Data recorded in tables (3-5) illustrated that the incidence of *Salmonella* in frozen meat was 6.4%.Table (3) showed that the most prevalent *Salmonella* serotypes isolated were *S. Infantis* and *S. Hafnia* imported from South Africa in winter 2014 and summer 2014 respectively, while imported chilled meat from USA and South Africa was free from *Salmonella*. Table (4) revealed that the most prevalent isolated serotype was *S.Typhimurium* imported from Australia in winter 2015.Table (5) showed that the most prevalent isolated serotype was

S.Newport imported from USA in summer 2014.The obtained results were higher that recorded by **Ahmed and Shimamoto (2013).** High incidence of *Salmonella* from imported frozen meat (4 consignments) in the present study can be attributed to contaminated bovine meat increased between slaughterhouse and packaging, moreover contamination through storage.

Seasonal incidence of *Salmonella*

The results of seasonal sero-prevalence of *Salmonella* were presented in figure (1). It was observed that prevalence rate of *Salmonella* higher (60%) in summer season and decreased in winter (40%) but lower in autumn (20%), absence in spring. The obtained results were closely similar to that reported by **Williams *etal*(2013).**The seasonal variation of sero-prevalence might be due to epidemiological factors and environmental changes.

Conclusively, Concerning *Salmonella* in imported meat, it must be change Minister of Health No. 298 for the year 1980, imported meat consignments is fit for human consumption in case of proven wither as result of bacteriological representative samples of these consignments from meat tests as positive for *Salmonella* by 10%.

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الملخص العربي

تم تقييم الحجر الصحي ضد السالمونيلا عن طريق مطار القاهرة الدولي من يوليو 2014 حتى يوليو 2015 و من يناير 2014 حتى يوليو 2015 للخيل المستوردة واللحوم؛ على التوالي. وشملت العينات البرازية من الخيول المستوردة (ن = 90)، واللحوم المبردة المستوردة (ن = 67) واستوردت اللحوم المجمدة (ن = 63). استيراد الخيول من بلجيكا وإنجلترا وألمانيا وفرنسا والإمارات والكويت خالية من السالمونيلا . على الرغم من أن 1.5٪ من اللحوم المبردة المستوردة كانت إيجابية لالسالمونيلا. كان المصلي الأكثر انتشارا معزولة S. كنتاكي، المستوردة من أستراليا في الصيف 2014، في حين كانت 6.4٪ من اللحوم المجمدة المستوردة إيجابية لالسالمونيلا. كان المصلي الأكثر انتشارا معزولة S. الطفلية، S. الهافنية، S.Typhimurim وS.Newport المستوردة في فصل الشتاء 2014، صيف 2014 (جنوب افريقيا)، شتاء 2015 (أستراليا) وصيف 2014 (الولايات المتحدة الامريكيه) على التوالي. معدل انتشار السالمونيلا أعلى (60٪) في موسم الصيف وتنخفض في فصل الشتاء (40٪) ولكن أقل من ذلك في فصل الخريف (20٪)، وغياب في فصل الربيع. كان S. Typhimurim حساسة لل Florfenicol ، جنتاميسين والستربتوميسين.