Animal Health Research Institute In Assiut

ISOLATION AND SEROTYPING OF SALMONELLA SPECIES FROM RAW MILK OF COWS, BUFFALOES AND SHEEP IN ASSIUT GOVERNORATE

(With 2 Tables)

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(Received at 9/12/2007)

عزل وتصنيف ميكروب السالمونيلا من اللبن الخام فى البقر والجاموس والغنم فى محافظة أسيوط

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جُمعت مائة وخمسون عينة عشوائية من ألبان البقر والجاموس والغنم (٥٠ عينة من كل نوع) من منازل الفلاحين ومحلات بيع اللبن فى محافظة أسيوط وذلك لمعرفة مدى تلوث اللبن بميكروب السالمونيلا وقد تم عزل الميكروب وإجراء الاختبارات البيوكيميائية وعمل اختبار الحساسية للعترات المعزولة بمعمل بحوث صحة الحيوان بأسيوط ولكن تم تصنيف الميكروب سيرولوجيا بكلية الطب جامعة أسيوط. وقد أظهرت النتائج أن ٢٥ عينة كانت إيجابية لميكروب السالمونيلا للبن الخام للحيوانات المختلفة أى بمعدل ٦,١٧% وقد كانت فى البقر والجاموس والأغنام بنسبة ٢% ، ٢٠% ، ٢٨% على التوالى. وقد أسفرت النتائج أيضاً على أن سالمونيلا جالنيرم هى السائدة على باقى العترات المعزولة من اللبن وكانت بنسبة ١٠% منهم ١٠ عترات بنسبة ٢٠% فى الجاموس و٥ عترات بنسبة ١٠% فى الأغنام ولكن سالمونيلا أنتراتيدس كانت بنسبة ٦% منهم ٢% فى البقر و١٦% فى الأغنام. أما سالمونيلا تايفاى عُزلت فقط من ألبان الأغنام وكانت بمعدل عينة واحدة بنسبة ٢%. كانت نتائج اختبار الحساسية لثمانية أنواع من المضادات الحيوية لكل أنواع عترات السالمونيلا المعزولة حساسة بدرجة عالية للنورفلوكساسين وبدرجة متوسطة للإستربتومايسين وبدرجة أقل للأمبسيلين، سيفاتكسيم كلورامفنيكول وسيفادروكسيل وريفامين ماعدا سالمونيلا جالنيرم كانت حساسة بدرجة متوسطة للجنتامسين وبدرجة أقل حساسية لكل من كلورامفنيكول وسيفادروكسيل.

SUMMARY

One hundred and fifty random samples of raw milk from cows, Buffaloes and Sheep (50 samples of each) were collected from different farmer's houses and dairy shops in Assiut Governorate to be examined for the presence of salmonella organisms on pre – enrichment and enrichment then plating on selective agar media, the Biochemical tests and serological tests were applied. The obtained results revealed that, the

salmonella Spp. could be detected from raw milk of different animals were 25 (6.17%) positive samples, 1 (2%), 10 (20%) and 14 (28%) for cows, buffaloes and sheep milk, respectively. Serotyping of the isolated salmonella spp. revealed that, the Salmonella gallinarum was the most prevalent species among the salmonalla spp. 15 isolates (10%) 10 (20%) from buffaloes and 5 (10%) from sheep. Salmonella enteritidis was isolated from 9 samples, 1 (2%) from cows and 8 (16%) from sheep but only one sample of sheep milk was positive for salmonella typhi. All types of the isolated salmonella were highly sensitive to Norofloxacin of the tested antiblotics and moderately sensitive to streptomycin and weekly sensitive to Gentamycin, Rifampin, Ampicillin, Cefotaxime, cefadroxil and chloramphenicol. However salmonella gallinerum was moderately sensitive to Gentamycin and resistant to Cefadroxil and Chloramphenical.

Key words:

INTRODUCTION

Salmonella is the most important food borne pathogen in the world. Food borne diarrheal diseases caused by salmonellae affect more than 300 million humans worldwide (Chevrier *et al.,* 1995).

Salmonella is a leading cause of gastroenteritis in humans. Each year, approximately 40.000 cases of salmonelosis are reported in the united states, and it is estimated that 1.000 people die from acute salmonellosis (Anonymous, 2003). The majority of cases are due to the consumption of contaminated animal products such as poultry eggs, raw meats, raw milk and other dairy products that have not been pasteurized or have been handled inappropriately (Centers for Disease Control and Prevention, 1996. and 2000).

Milk and milk products have been identified as the vehicle for transmission in approximately 5% of Salmonelosis cases, although the sources of infection remain unidentified in most cases (Centers for Disease Control and Prevention, 2000) Salmonellosis is commonly diagnosed in dairy cows and calves, and the presence of salmonella on dairy farms has been well documented, (losinqer, *et al.,* 1995; wells, *et al.,* 2001 and Huston *et al.,* 2002a).

Salmonellosis in cattle can result in gastritis, abortion, decreased milk production or even death but fecal shedding of salmonella by asymptomatic animals has also been observed (Huston *et al.,* 2002b).

Although there is evidence of salmonella sheding by the mammary gland (Smith *et al.,* 1989; Radke *et al.,* 2002). Fecal contamination is also likely a major source of contamination of raw milk.

Rohrbach *et al.* (1992); Steele *et al.* (1997); Jayarao *et al.* (2001) and Murinda *et al.* (2002) reported that 0.17% of bulk tank milk was contaminated with salmonellae.

Kivanc, *et al.* (1992) detected salmonellae–shigella in two milk samples obtained from Eskishir markets. Wallaa (2004) detected salmonella (6%) species in milk and some milk products in Assiut city, but Muehlherr, *et al.* (2003) and Ekici, *et al.* (2004) could not detecte salmonella spp. from cows, goats and ewes milk.

Although the majority of milk is consumed as pasteurized milk, many farmer familles drink raw milk (Jayarao, and Henning, 2001).

Soft Mexican – style cheeses are often made with unpasteurized milk, and several reported outbreaks of salmonellosis have resulted from the consumption of such chesses (Cody *et al.,* 1999 and Villar *et al.,* 1999).

The purpose of this study:

- Isolation and identification of salmonella Spp. from raw milk.
- Serotyping of the isolated salmonella Spp.
- Antimicrobial susceptibility of the isolated salmonella Spp. were done.

MATERIALS and METHODS

1- Collection of samples:

One hundred and fifty samples of raw milk from cows, buffaloes and sheep (50 samples of each). All samples were collected from Assiut city in clean, dry and sterile containers. Collected samples were transferred to the laboratory as soon as possible to be examined.

2- Prepration of samples:

Every milk sample was tested for detection of heat treatment by storch test according to Lampert, 1975.

3- Methods of isolation of salmonella species:

The methods of isolation used in this study were according to Andrews and Hammack, 2001 and involved four basic steps: Preenrichment, selective enrichment, selective plating and biochemical testing.

4- Serological identification:

Serological identification by using the slide agglutination technique with polyvalent somatic D and H tests. The applied technique was recommended by Edwards and Ewing (1972) and International Commission on Microbiological Specification for Foods (1978).

5- Antibiotics sensitivity test:

The applied method and inhibitors zones were measured and recorded according to (Quinn, *et al.*, 1994).

N.b. serotyping of isolated salmonella was performed by the department of bacteriology, Faculty of Medicine.

RESULTS

Table 1: Incidence of salmonella and salmonella serotypes from different raw milk samples

Examined samples		No. of positive samples		Salmonella serotypes					
				S. typhi		S.enteritldis		S. gallinarum	
	No.	No.	%	No.	%	No.	%	No.	%
Cows	50	1	2%	-	-	1	2%	-	-
Buffaloes	50	10	20%	-	-	-	-	10	20%
Sheeps	50	14	28%	1	2%	8	16%	5	10%
Total	150	25	16.7%	1	0.7%	9	6%	15	10%

Table 2: Sensitivity test of the isolated salmonella serotypes.

Antibiotics	S. typhi	S. enteritidis	S. gallinarum
1- Norafloxacin $(NOR)_{10}$	+++	+++	+++
2- Chloramphenicol (C_{30})	+	+	-
3- CEfadroxil $(CFR)_{30}$	+	+	-
4- Ampicillin $(AM)_{10}$	+	+	+
5- Rifampin $(RA)_5$	+	+	+
6- Gentamycin $(CN)_{10}$	+	+	++
7- Tetracycline $(TC)_{30}$	+	+	+
8- Streptomycin (S_{10})	++	++	++
9- Cefotaxime (CTX)	+	+	+

N.b.

(-) = Resistant (++) = Moderately sensitive

(+) = weak sensitive (+++) = highly sensitive

DISCUSSION

Salmonella in dairy farms has been well documented (Huston *et al.*, 2002). Milk and milk products have been identified as the vehicle for transmission in approximately 5% of salmonellosis cases (Centers for Disease control and Prevention 2000).

The summarized data in Table. 1 show that the genus salmonella could be isolated with varying percentages from raw milk samples of cows, buffaloes and sheep in a percentage of 20, 2 and 28% respectively. Raw milk samples of sheep constitute the highest rate of contamination 28%. (2%) salmonella typhi, 16% salmonella enteritidis and 10% salmonella gallinarum. This may be related to the more relaxed microbiological standards for the production and distribution of sheep's milk than those of cow's milk which has stringent hygiene and quality regulations.

Salmonella gains acess to sheep's milk either by fecal contamination or by direct excretion from the udder into milk (Rampling 1996). Sheep's milk play an important role in transmitting more pathogens including food poisoning micro organisms due to consumption of raw milk (Foster *et al.*, 1983). So, dairy industries for sheep's become important elsewhere (Maxy, 1993). Scanty data till now obtained concerning sheep's milk according to diary base line studies.

The present study in Table (1) also shows that the incidence of salmonella isolated from raw buffaloe's milk was 20% and the result was higher than that obtained by Singh and Singh (1966), Rohrbach *et al.*, (1992) and Wallaa (2004); nearly to El Said, (2002). While, the results were in agreement with Sharma *et al.*, (1995) who failed to detect salmonella in raw milk. High incidence of salmonella in raw buffaloe's milk came in line with Snyder and Poland, (1990) who postulated that high amount of fat in food provides a protective barrier around the microbial cells and prevent their disintegration by stomach during ingestion. Table (1) also reveals low incidence of salmonella isolated from raw cows milk samples (2%). The results came in line with McEwen *et al.* (1988), lower than Wallaa, (2004) and higher than O'Donnell, (1995) and Steele *et al.* (1997).

Although Cullor, (1997) stated that salmonellae all widespread in the environment of dairy cows and their eradication is difficult. Raw buffaloe's and cow's milk may be contaminated by salmonella from the use of polluted water, dairy equipments and healthy dairy carriers. In addition, the organism may be eliminated with feces of workmen on the

farm and secreted in milk of buffaloes and cows during the febrile stage of clinical salmonellosis. Rampling (1996). As shown in Table (1) salmonella species could be identified was salmonella enteritidis from raw cow's milk (2%). The results came in hormoney with Anuszl (1980), WHO (1995) and Olsen *et al.* (2000) and Wallaa (2004) who recorded that salmonella enteritidis was a causative agent and it was responsible for 55% of food – borne disease outbreaks. Another species which is salmonella gallinarum that could be isolated from buffaloes's milk (20%), salmonella gallinarum usually produces no or very milk infection (NRC 1969).

The differences in prevalence of salmonella among herds might have been caused by differences in hygiene and management practices by different media used (International standard organization "ISO" 1993).

Table (2) concerning the antimicrobial susceptibility pointed out that Norfloxacen (NOR_{10}) is the most predominant antibiotic was sensitive by all salmonella serotypes isolated from cows, buffaloes and sheeps milk samples.

While, the isolates were moderately sensitive to streptomycin (S_{10}) as reported by Farid (1976) and in contrast to Sojka and Hudson (1976). At the same time the isolates were weakly sensitive to Ampicillin (AM_{10}), Rifampin (RA_5) and Cefotaxime (CT_{30}) as reported by Sojka and Hudson (1976). Zein El Abden *et al.* (1966) stated that isolates from buffaloes were similar to those found in cattle. Results tabulated in Table (2) show that isolates that were initially identified biochemically as salmonella strains and subjected to serological identification were salmonella typhi (1) 2% from sheep's milk only, salmonella enteritidis (1) 2% and (8) 16% from cow's and sheep's milk samples, respectively. While, salmonella gallinarum was identified serologically from (10) 20% and (5) 10% of buffaloe's and sheep's samples respectively. Wallaa (2004) delt with serological identification of salmonellae.

In conclusion: The results obtained in this study reflect the importance of hygienic measures adopted during milking and handing by attendants to safeguard consumers from being infected by salmonellae to eliminate potentially of occurring hazard arizing from microbial contamination.

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