Orginal article

Incidence and Antimicrobial Susceptibility of *Salmonella* Carrier among Apparently Healthy Camels in Some Libyan Regions

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Abstract

Our study were planned to investigate the incidence of *salmonella* species among apparently healthy camels. One hundred and twenty one (121) samples were collected from camel's feces (79 from Egdabya and 42 from El-mackeli) in Libya. Bacteriological examination of samples showed that the incidence of positive fecal samples were 16 (13.2%) according to their culture and morphological characteristics. Biochemical and serological identification revealed the following serotypes: six of S. *typhimurium* with incidence of (37.5%), four of *S. entretids* (25%), three of *S. frintrop*) (18.8%), in addition to three isolates were not identified. Anti-biotic sensitivity of isolated serotype showed that serovars were most sensitive to Ampicillin, Amoxicillin/Clavulanic acid (all isolates) and Chloramphenicol which could be used for treatment of salmonellosis in camels. The epidemiology and zoonotic significance of *salmonella* infection were discussed in

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Introduction

Salmonellosis occurs in most countries and affects all animal species. Infection with *Salmonella* species in camel has been described by various authors since 1912(Wernery and Kaaden, 2002**)**.Acute enteritis due to salmonellosisis common both in camel calves and adult with diarrhea which may develop into severe hemorrhagic enteritis, while the chronic formis more prevalent in adult camels with symptoms of persistent diarrhea, fever, emaciation and poor response to treatment. Septicemia is a syndrome seen in newborn calves up to six months of age(Al-Ruwaili *et al*, 2012).

Calves develop fever and depression and may die within 48 hours. Animals that recover often have the bacteria in their faeces for a long time, thus become a source of infection for other animals (Bakhat et al.,2003). The breeding of domestic animals with increased resistance to *Salmonella* spp.is an attractive option (Wigley, 2004).

The basic virulence strategy common to *Salmonella* spp. is to invade the intestinal mucosa and multiply in the gut-associated lymphoid tissue. From the infected intestinal tissue the pathogens are drained to the regional lymph nodes, where macrophages that line the lymphatic sinuses form a first effective barrier to prevent further spread (Baumler *et al.* 1997).

Control methods currently or previously applied often have short comings. these include increased production costs associated with the use of vaccination, public health issues in the use of prophylactic antibiotics potentially leading to development of antibiotic resistant strains of pathogenic bacteria or only partially effective measures such as the use of pro biotic bacteria in competitive exclusion (Wigley 2004).This study aimed at determining the serotypes, antimicrobial profiles, and the incidence of *Salmonella* species among apparently healthy camels in Libya.

Material and Methods

Sample collection

Fecal sample were collected from 79 camels from Egdabya and 42 camels from El-mackeli without clinical science of diarrhea. These camels are not treated with antibiotic. A single specimen was obtained from each animal.

Isolation of Salmonella Species

According to Harvey and Price (1974) each fecal specimen was homogenized by mixing with a tooth pick, then a pea sized amount added to 10 ml of selenite F broth in screw capped bottle and incubated at 37 ºC for 24 hours, subcultures were then made into plates of MacConky agar (Merck, Germany), Xyloselysine Desoxycholate (XLD) agar (Oxoid), *Salmonella* agar (Merck), *Salmonella Shigella* (SS) agar (Oxoid) and incubated at 37 ºC for another 24 hour. Pure colonies were sub cultured into nutrient agar and incubated for 24 hours.

Identification of Salmonella Species

The cultures on nutrient agar plates were subjected to gram staining, motility, urease production, hydrogen sulphide production and citrate utilization tests. All gram negative rod shaped motile, urease negative isolates that produced acid on triple sugar iron agar slants and able to utilize citrate as sole carbon source were identified as species of the genus *salmonella* .The

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plate cultures were examined for suspected colonies and confirmed by API-20E (Biomereux).

Serological identification of Salmonella species

Serologic identification of Salmonella species was determined in Animal Health Research Institute (Dokki, Egypt**)** and performed by slide agglutination test with (Andrews *et al.* 2005). An agglutination test was performed on a clean glass slide. The slide was divided into sections with a wax pencil and one small drop of physiological saline was placed in each test section on the slide. By using a sterile inoculating loop a portion of growth from the surface of TSI agar was removed and emulsified in each drop of physiological Saline on the slide.

It was then mixed thoroughly to create a moderately milky suspension. A bent inoculating loop was used to pick a small drop of antiserum and transferred to one of the suspensions; the second suspension served as the control (usually approximately equal volume of antiserum and growth suspension was mixed).

The suspension and antiserum was mixed very well and then the slide was rocked to observe for autoagglutination (agglutination is more visible if the slides are observed under a bright light and over a black background). If the reaction is positive, clumping will appear within 30 to 60 seconds. The saline suspension (control) was examined carefully to ensure that it was even and did not show clumping resulting from auto agglutination.

Antimicrobial susceptibility

All *Salmonella* strains were tested for susceptibility to a panel of 9 antimicrobial disks by the disc–agar diffusion method on Mueller -Hinton agar (oxoid), following the National Committee on Clinical Laboratory Standard. Each *Salmonella* serovar was cultured on Nutrient broth and incubated at 37C for 12 hours. Adjusted turbidity sample was then spread over the surface of Mueller Hinton agar.

Antibiotic discs were disposed on the surface of inoculated agar media aseptically and incubated at 37ºC for 18-20 hours. The inhibition zones of each disk were measured and the results were interpreted based on comparison to standards. Antibiotic discs used were: ampicillin (10), amoxicillin/clavulanic acid (30), cloxacillin (30), gentamycin (10), streptomycin (10), chloramphenicol (10), oxytetracycline (30), neomycin (30) and cephalexin (30).

Results and Discussion

Out of 121 examined samples 16 *Salmonella* isolates (13.2%) were recovered from feces. The recorded infection rate of *Salmonella* spp. in camel feces was near to those reported by (Molla *et al.* 2004),whose result was15.1% and (Al-Ruwaili *et al.*, 2012)whose record a rate of14.7%. On other hand, a higher infection rate with salmonellosis in camel (11/15, 73.3%) was reported by (Pegram and Tareke 1981).This study recorded the incidence of salmonellosis in camels (13.2%) which is higher than that found in UAE (4.3%) by (Wenery1992). The isolation of *Salmonella* spp. from camel fecal samples may probably due to fecal-oral contamination of feedstuffs, feeding surfaces, water troughs and equipment from carrier human or animals sources.

Results of serotyping of 16 isolated *Salmonella spp.* revealed that *S. typhimurium* represented the higher incidence, it was recovered from 6 samples (37.5%), followed by *S.entritidis* 4 (25%), *S.frintrop* 3(18.8%) in addition to three isolates could not be identified) Table. 1).These results were similar to the finding of (Faye *et al.* 1997). They reported that the most important *Salmonella* serotypes in camels were *S.typhimurium, S.entritidis, S.kentuky* and *S.saint-paul*. Also agreed with the results of (Wernery and Kaaden 2002).They identified 69 different *Salmonella* serotypes isolated from camels all over the countries they studied including *S.typhimurium, S.entritidis, S.muenchen, S.bovismorbificans* and *S.derby*.

From zoonotic point of view Matofari and colleagues (2007) mentioned that healthy camels can be carriers *of Salmonella* spp. which could be isolated from their feces and lymph nodes. Camels that are chronic carriers of Salmonella may present as human health hazard through consumption of camel's products like milk (Matofari *et al.* 2007**)**. Salmonellosis in camels was reported in Sudan (Curason 1998), United States of America (Bruner and Moran 1949**)** and from Somalia **(**Cheyna *et al.*1977).

The literature showed that *Salmonella* spp. could be present in camels of all ages (Wernery 1992; Salih *et al.*1998; Berada *et al.* 2000). Camels and their products could be a potential reservoir for *Salmonella*s pp. not only to the remaining camels but also to human and other animal species (Morpeth *et al.* 2009).Continuous surveillance studies for *Salmonella* in human and animals are important since new *Salmonella* serovars are emerging yearly and serotyping is very important to the epidemiological studies (Kim, 2010; Al-Ruwaili *et al.*, 2012).

Concerning the susceptibility of isolated Salmonella serovars to antibiotics is presented in Table (2) .The highest sensitivity rate was recorded to ampicillin (100%), amoxicillin / clavulanic acid (100%) and chloramphenicol (93.75%) and the highest resistance rate was recorded to oxytetracycline (100%), Neomycin (93.75%) and cloxacillin (81.25%).

Some isolates were intermediate to gentamicin (75%), Streptomycin (68.75%) and Cephalexin (87.5%). Although antibiotic therapy is important in treatment of salmonellosis in most endemic countries, antibiotics such as ampicillin, amoxicillin/clavulanic and chloramphenicol have been banned in Asia and Sub Saharan Africa (Amyes and Gupta 2002). Not all

Table 1. Prevalence, serotyping of *Salmonella* spp. isolated from Agdabia and El-macheli Region

Region	No. of fecal samples	Positive		Serotypes
		No.	%	
Egdabya	79	11	13.9	*S. enteritidis* (4), S. *Frintrop* (3) and *S.* typhimuriu (3) m one strain could not be identified
El-macheli	42	5	11.9	*S. typhimurium* (2), *S. Enteritidis* (2), and two strains could not be identified

antimicrobials at the concentration required to be effective are completely non toxic to human cells. However, the degree of susceptibility in determining the length of therapy and choice of cheaper antimicrobial agents with less side effects (Edward and Ewing 2003; Ngozi and Onyenekwe 2003) The high antibiotic resistance demonstrated by these isolates in our study is correlated with the high level of antimicrobial resistance in Enterobacteria, in fecal flora as well as in clinical isolates reported by (Velonakis *et al.* 2001; Aarestrup 2005).

Table 2. Antimicrobial susceptibility of *Salmonella* spp. (n = 16) by agar disc diffusion method.

Antimicrobial agent	Disc Concentration	Susceptible		Intermediate		Resistant	
		No	%	No	%	No	%
Ampicilline	10 mg	16	100	0	0	0	0
Amoxicillin/Clavulanic acid	AMC 30 mg	16	100	0	0	0	0
Streptomycin	S 10 mg	5	31.25	11	68.75	0	0
Neomycin	N 30 mg	1	6.25	0	0	15	93.75
Cloxacillin	CX 30 mg	2	12.5	1	6.25	13	81.25
Gentamycin	G 10 mg	2	12.5	12	75	2	12.5
Chloramphenicol	C 10 mg	15	93.75	0	0	1	6.25
Oxitetracycline	30 mg	0	0	0	0	16	100
Cephalexin	CL 30 mg	0	0	14	87.5	2	12.5

Results are expressed as a percentage of (n = 16 (*Salmonella* spp. isolates susceptible, intermediate/moderately susceptible and resistant, respectively, for each antimicrobial.

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