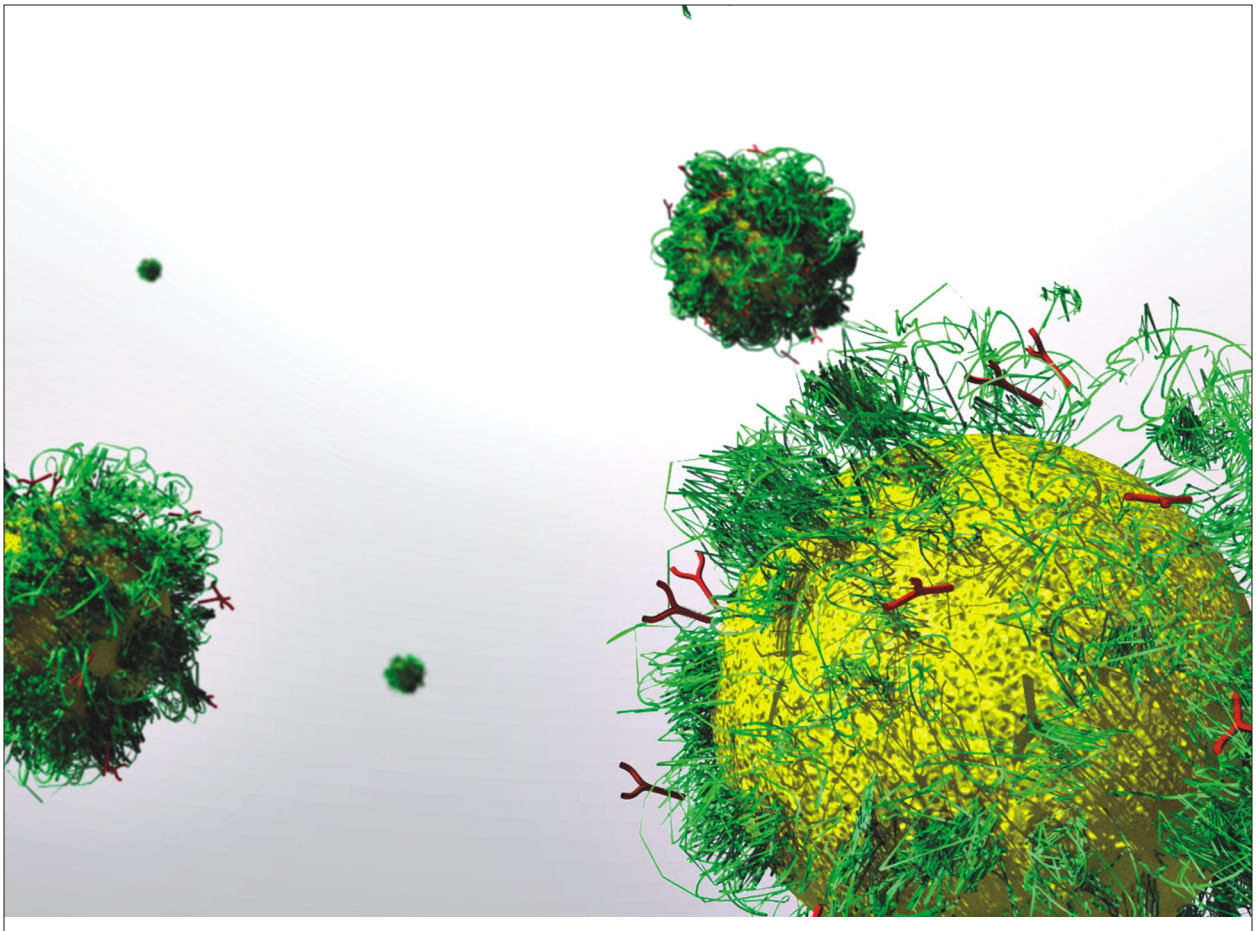

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Survey on *Salmonella* Infection Rate in Horses of Some Horse Riding Clubs Southern Iran

[1]Ali Ghorbani Ranjbry, [1,2]Nazanin Ghorbani Ranjbry and [2]Zahra Ghorbani Ranjbry
[1]Young Researchers Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[2]Khormshahar Marine Science, Technology University, Iran

Corresponding Author: Ali Ghorbani Ranjbry, Young Researchers Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran

ABSTRACT

In this survey which was taken in 7 horse riding clubs in Ahvaz, the rate of *Salmonella* infection in apparently healthy horses were investigated. The Fecal samples were collected from 129 horses and cultured between April 2011 to January 2012. The sampling and culture were done 5 times in two week intervals for each horse. Isolation and identification of *Salmonella* from fecal samples were done according to routine bacteriological and PCR methods. The results of this survey showed that 3 (2.32%) horse was infected with *Salmonella* and it was identified as *Salmonella enteritidis*. Among the separated *Salmonella*s 93% were resist to Ampicillin, 16.5% to Tetracycline and 40.5% to Kanamycin, but all of them were sensitive to Gentamicin in this antibiotic resistance test.

Key words: *Salmonella* spp., *Salmonella enteritidis*, drug resistance, horse

INTRODUCTION

Salmonella is an important foodborne pathogen world-wide. A recent study estimated that approx. 93.8 (95% Confidence Interval: 61.8-163.6) million human cases of gastroenteritis and 155 000 (95% Confidence Interval: 39 000-303 000) deaths occur due to *Salmonella* infection around the world each year (Majowicz *et al*., 2010). In the USA alone, *Salmonella* causes an estimated 1.4 million human cases, 15 000 hospitalizations and more than 400 deaths annually (Voetsch *et al*., 2004; Mead *et al*., 1999). However, only a fraction of cases is reported and in the USA, only an estimated 1-5% of cases are laboratory confirmed and reported to the Centers for Disease Control and Prevention (CDC) (Chalker and Blaser, 1988). In 2006, the national case rate in the USA equaled 13.6 reported cases per 100000 population per year (Chalker and Blaser, 1988). Rates varied considerably by geographic region, with estimates particularly high in the Mid-Atlantic and New England States. This heterogeneity is likely in part due to differences in reporting. Differences in salmonellosis case rates between geographically and socio-economically similar USA states have been documented, with rates differing by as much as 200% between neighboring states (Chalker and Blaser, 1988). Similarly, of the 168929 human cases reported in the European Union (EU) during 2005, 31% stemmed from Germany even though less than 20% of the EU's population resides in Germany, again suggesting reporting differences (EFSA, 2008; Lanzieri, 2008). In 1999, non-typhoidal *Salmonella* infections in the USA were estimated t contribute 10% of foodborne human illnesses, 26% of hospitalizations and 31% of deaths

attributable to infections by known foodborne pathogens, thereby ranking first among all bacterial foodborne pathogens in hospitalizations and deaths and second after Campylobacter in the number of illnesses (Mead *et al*., 1999). In 2009, *Salmonella* was the most commonly reported bacteriological agent of human foodborne disease in the USA, causing approx. 44% of confirmed foodborne bacterial infections (CDC, 2010). More than 20% of human clinical *Salmonella* isolates in the USA are obtained from children under the age of 5 years, emphasizing the great importance of this age group (D'Aoust, 1978; CDC, 2008). Approx. 1% of *Salmonella* cases are thought to require hospitalization (Doyle *et al*., 2009). However, due to the high prevalence of *Salmonella* infections, the Economic Research Service of the United States Department of Agriculture (USDA, 2010). Salmonellosis is an important disease of horses. Equine mortality rates vary depending on host age, predisposing factors and potentially the *Salmonella* serotype involved (Wenkoff, 1973). Mortalities as high as 40-60% have been reported but in general, mortality appears to be considerably lower (Begg *et al*., 1988; Smith *et al*., 1980). In most cases, animals present with profuse, watery and malodorous diarrhea, frequently associated with abdominal pain and endotoxemia. Fever, dehydration and depression are common and in severe cases these symptoms are accompanied by colic, gastric reflux, cardiovascular shock or coagulopathies. However, the severity of disease can vary considerably and, in animals of the same age group, may range from severe to asymptomatic (Roberts and O'Boyle, 1982). Both peracute and chronic forms of disease are common and convalescent carriers may shed *Salmonella* for months, but a carrier state does not appear to develop in all instances (Smith *et al*., 1980; Morse *et al*., 1976; McCain and Powell, 1990). Disease may also manifest without gastrointestinal signs. Some serotypes appear to result more frequently in systemic disease than others, but the underlying mechanisms are still incompletely understood (Ikeda *et al*., 1986). Respiratory forms are comparably frequent and systemic forms of infection are commonly associated with arthritis, osteomyelitis, or soft-tissue abscesses (Blikslager *et al*., 1991; Platt, 1973). Foals, pregnant mares and immune compromised horses are at a heightened risk of infection and among foals, *Salmonella*-associated meningoencephalitis has been described (Ernst *et al*., 2004). Abortions due to *Salmonella* cause important economic losses on stud farms (Donahue, 1986; Kumar and Gupta, 1972). Numerous studies have focused on horses in equine hospitals, with apparent prevalence estimates ranging from 1.8-18%; Anderson and Lee, however, report isolating *Salmonella* from 26.6% of slaughter horses (Anderson and Lee, 1976; Roberts and O'Boyle, 1981). This study Survey on *Salmonella* infection rate in horses of some horse riding clubs Southern Iran took place.

MATERIALS AND METHODS

The Fecal samples were collected from 129 horses and cultured between April 2011 to January 2012. The sampling and culture were done 5 times in two week intervals for each horse. Isolation and identification of *Salmonella* from fecal samples were done according to routine bacteriological and PCR methods.

Recognition of *Salmonella* and serotype enteritidis *Salmonella typhimurium* bacteria by PCR test: For DNA extraction of identified *Salmonella* in above, a colony suspensions in sterile distilled water were prepared. The suspension was centrifuged for 5 min at 18°C with a speed of

Table 1: Oligonucleotide sequences used as primers in polymerase chain reaction (PCR)

Primer	Sequence (´3-´5)	Amplicon fragment (bp)
InvA-1	TTG TTA CGG CTA TTT TGA CCA	
InvA-2	CTG ACT GCT ACC TTG CTG ATG	521
SefA-1	GCA GCG GTT ACT ATT GCA GC	
SefA-2	TGT GAC ACG GAC ATT TAG CG	330
PefA-1	TTC CAT TAT TGC ACT GGG TG	
PefA-2	GGC ATC TTT CGC TGT GGC TT	479

Table 2: Pattern of drug resistance of *Salmonella* isolated from the native eggs

Result	Ampicillin	Tetracycline	Kanamycin	Norfloxacin
Sensitivity	0	42	20	100
Semi-critical	7	41/5	39/5	0
Resistance	93	16/5	40/5	0
Total	100	100	100	100

14,000 rpm and bacterial genome was extracted from the precipitated, by phenol-chloroform bacterial genome method. Genome by using specific primers of invasion gene used for PCR to detect *Salmonella*. To determine the serotype, the primers of Fymbrya gene (sefA) for *Salmonella enteritidis* and primers of Virulence Fimbriae (pefA) for *Salmonella typhimurium* were used in PCR (Table 1). PCR products on agarose gel 1.5% electrophoresis and 100 bases pair were used to determine the molecular weight of DNA fragments. Gel stained with ethidium bromide solution which has a Fleur Sans color. UV radiation device used for observing bright bands under the ultraviolet light (Cortez *et al*., 2006)

Antimicrobial resistance: To determine the sensitivity of isolated *Salmonella* to the antibacterial compounds, a disk agar diffusion method was used. First, colonies in Moler Hilton liquid environment was reached to half Mcforlan, then a culture was provided from it on the agar Muler Hilton culture environment and simultaneously a disk containing antibiotic was placed (from the Padtan teb company on the culture environment). After 24 h incubation at 37°C, *Salmonella* growth inhibitions ring were determined. For antibiogram tests, four antibiotics disks containing: Ampicillin (10 μg), Tetracycline (30 μg), Kanamycin (30 μg) and Gentamicin (10 μg) were used (Table 2). The result of *Salmonella* isolation from eggs estimated by the eggs contamination percentage. the results of determining *Salmonella* sensitivity to anti-bacterial effect also on the base of the percentage were stimated in 3 sensitive, semi-sensitive and-resistant level. (based on the company's table of Padtanteb from the number of *Salmonella* samples) (using standard Kirby and Bauer technique) (Adesiym *et al*., 2007; Quinn *et al*., 1994).

RESULTS

The results of this survey showed that 3 (2.32%) horse was infected with *Salmonella* and it was identified as *Salmonella enteritidis* (Fig. 1). In pattern of studding drug resistance of isolated *Salmonella*, resistance rate to the antibiotic of Ampicillin %93, Kanamycin %40.5 and Tetracycline %16.5, respectively while there was not observed resistant sample to Gentamicin. Regarding to antibacterial drug used, the most sensitive was observed for Gentamicin (Table 2) which is due to not using these antibiotics in the area and findings no resistance to it.

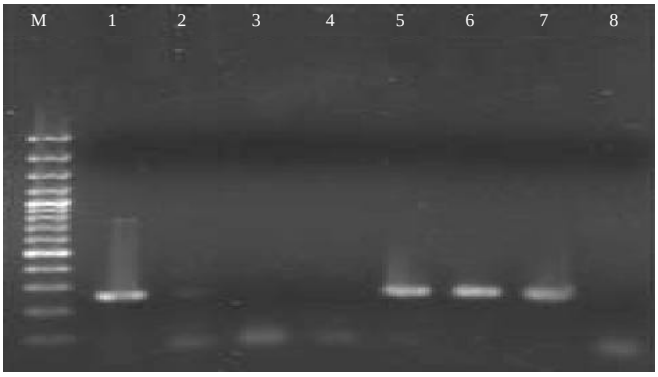

Fig. 1: Detection of *Salmonella enteritidis*, Row M: Indicator of molecular weight DNA; Row 1: positive control (standard strain of *Salmonella enteritidis*,) rows of horse contaminated with *Salmonella entraytdys* 5, 6, 7 rows of horse-free from *Salmonella enteritidis*, row 8 negative control

DISCUSSION

The results of this survey showed that 3 (2.32%) horse was infected with *Salmonella* and it was identified as *Salmonella enteritidis*; Most of the isolates were *Salmonella typhimurium*, the serovar most commonly reported in horses, in which MRD is increasingly prevalent (Van duijkeren *et al*., 2002) and which is responsible for both human and animal outbreaks in Europe (Van duijkeren *et al*., 2002; Horby *et al*., 2003). The use of PFGE for identifying genetic relatedness and possible clustering of MDR *Salmonella typhimurium* DT104 in animals is well-established (Zheng *et al*. 2011) and susceptibility testing is recognized as an important component of any preliminary identification of MDR *Salmonella* strains especially *Salmonella typhimurium* DT104 (Thorsteinsdottir *et al*., 2007). The resistance patterns identified in our isolates are similar to other studies of MDR *Salmonella* DT104 in horses, where the resistance pattern ACSSuT is the most widely reported (Van Duijkeren *et al*., 2002). Many studies have shown the increasing prevalence of antibiotic resistance in *Salmonella typhimurium* in both farm animals and humans (Zheng *et al*., 2011; Dargatz and Traub-Dargatz, 2004). All the *Salmonella* in this study were resistant to at least one antibiotic and many were multidrug resistant. Two distinctive resistance phenotypes were noticed among the multidrug resistant isolates: ACTTrSuFlo and ACTSuFlo. These were similar to the resistance profiles of *Salmonella typhimurium* from two human outbreaks in England in 2000 (Horby *et al*., 2003) and also were attributed to the consumption of contaminated food of equine origin in Netherlands (Van duijkeren *et al*., 2002). Recent increases in MDR-*Salmonella* have been of particular importance owing to the emergence of the multi-resistant *Salmonella typhimurium* DT104 in both humans and animals (Lawson *et al*., 2004; Rayamajhi *et al*., 2008). Although MDR-*Salmonella* types, especially DT104, have been associated with meat consumption and contact with infected animals (Espie *et al*., 2005) (Doorduyn *et al*., 2008), our findings also suggest that horse faeces could be a potential source of human MDR *Salmonella* infections.

In pattern of studding drug resistance of isolated *Salmonella*, resistance rate to the antibiotic of Ampicillin %93, Kanamycin %40/5 and Tetracycline %16/5, respectively while there was not observed resistant sample to Gentamicin. Regarding to antibacterial drug used, the most sensitive

was observed for Gentamicin (Table 2) which is due to not using these antibiotics in the area and findings no resistance to it. Having no complete sensitivity to Tetracycline and Kanamycin can result of excessive use of these antibiotics in the treatment. Then resistance to these antibiotic is not unexpected. Ampicillin resistance may be due to more effect of these antibiotics on Gram-positive bacteria than Gram-negative bacteria (Adams, 1995). Graziani *et al.* (2008) examined antibiotic sensitivity of isolated *Salmonella typhimurium* from humans and animals and also they determined the resistance of *Salmonella*, (which was isolated from poultry), to Ampicillin (3.54%), Gentamicin (2.3%), Kanamycin (85%), chloramphenicol (5.24%), Tetracycline (52.1%) and ciprofloxacin (0%) (39). Pan *et al.* (2009) studied Antibiotic resistance of *Salmonella antryka* which showed approximately 39/95% percent isolated bacteria showed high resistance, particularly against ampicillin (40.2%), Streptomycin (58%) and Tetracycline (58.9%). During the years, 1999-1962, all isolated *Salmonella* were sensitive to Norfloxacin, but during the years 2007-2000 showed low resistance (17.5) to Floxacin.

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