

STUDIES ON PREVALENCE AND RISK ASSOCIATED FACTORS OF ENTERIC *SALMONELLA* (*INVA* GENE) IN HORSES IN BIKANER[#]

T.C. Nayak*, R.K. Dedar¹, A. Chahar and Savita²

Department of Epidemiology and Preventive Veterinary Medicine, College of Veterinary and Animal Science
Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

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ABSTRACT

Salmonella is an important intestinal pathogen of many livestock species and human beings. It infects equines with or without demonstrating clinical illness. In present investigation one hundred sixty horses were screened for prevalence of enteric *Salmonella* at Bikaner. Horses were divided in to two groups; in group 1st faecal samples in triplet were taken from one hundred ten apparently healthy horses and in group 2nd faecal samples in triplet were taken from fifty hospitalized horses. Presence of *Salmonella InvA* gene by using polymerase chain reaction (PCR) in rectal swab cultured selenite broth was used as marker for salmonella shedding. Over all prevalence of enteric *Salmonella* in apparently healthy horses was found 2.72 per cent and 16 per cent in hospitalized horses. Antimicrobial treatment, history of diarrhoea and colic, history of laminitis, long distance transportation and gastrointestinal diseases were ascertained as risk factor for the gut colonisation of *Salmonella* and shedding.

Key words: Horses, *Salmonella* shedding, gastrointestinal tract diseases

Introduction

Salmonellosis is caused by enteric or systemic infection with bacteria of *Salmonella* spp. Horses can transiently shed *Salmonella* organisms in faeces without showing any clinical sign. These latent carriers can turn into a clinical case under stress condition. Case fatality rate of salmonellosis in equines is up to 60% (Radostits *et al.*, 2007). Veterinary teaching hospitals are at high risk of nosocomial salmonellosis infection in horses because of exposure of the hospital population to a common source of *Salmonella* organisms (e.g., contaminated feed) or by lateral transmission from infected patients. In some instances, these facilities have been forced to close temporarily (≥ 3 months) because of serious outbreaks of clinical disease caused by *Salmonella* infection in horses (Ernst *et al.*, 2004). Climate change is changing the pattern of occurrence of various infectious diseases in countries (Gale *et al.*, 2009).

Climate of Bikaner has been changed in last few decades (Chaudhary *et al.*, 2009) due to increased irrigation facilities and global climate changes. Environment temperature and humidity impact survival of *Salmonella* in the faeces, soil and pastures (Zhang *et al.*, 2010). So it was important to study the prevalence of *Salmonellae* and associated risk factors in the equine populations of Bikaner in changing climate conditions.

Materials and Methods

Collection of samples

Rectal swabs were taken from one hundred sixty horses in triplet over the period of 24 hours by using transport swab containing cary-blair medium. Out of these horses in group 1st, 330 rectal swabs were collected from 110 apparently healthy horses and in group 2nd, 150 rectal swabs were collected from 50 hospitalized horses in Bikaner area from different stud farms and veterinary hospitals. Clinical history of the animal during last 3 months and management practices were also recorded to assess risk factors associated with enteric *Salmonella* positive cases.

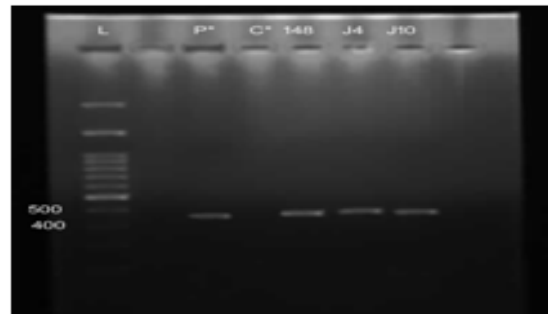


Fig. 1: Amplification of a 457bp fragment of *Salmonella* genus specific *InvA* gene in apparently healthy horses.

P* = +ve Control; C* = -ve Control; L = Ladder

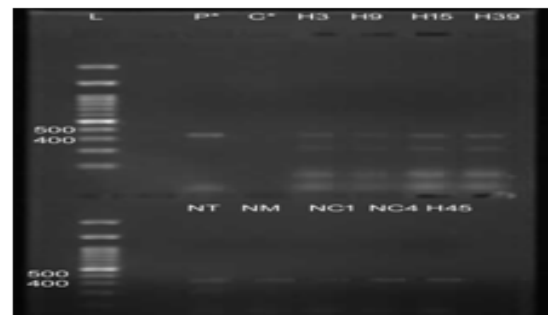


Fig. 2: Amplification of a 457bp fragment of *Salmonella* genus specific *InvA* gene in hospitalized.

P* = +ve Control; C* = -ve Control; L = Ladder

Sample processing

Rectal swabs were placed in to the selenite F enrichment broth. Cultures were incubated at 37°C for 36 hours under aerobic conditions. DNA was extracted using DNASure Blood Mini Kit NP-61107 Genetix Biotech Asia Pvt. Ltd (New Delhi). A highly conserved 457 base pair (b.p.) nucleotide sequence within the invasion gene (*invA*) of *Salmonella* spp. was targeted for amplification (Stone *et*

[#]Part of M.V.Sc. Thesis, Corresponding author Email: tsubhashnayak@gmail.com; ¹Scientist, ICAR-NRCE, Bikaner; ²Ph.D. Scholar, Department of Clinical Veterinary Medicine, Ethics and jurisprudence, CVAS, RAJUVAS, Bikaner

al., 1994). The sequence for forward primer was 5' TGC CTA CAA GCA TGAAAT GG 3' and for reverse primer was 5' AAA CTG GAC CAC GGT GAC AA 3'. The expected product size was 457 base pair (b.p.). The amplification was carried out in thermocycler with initial denaturation at 95°C for 2 min and 40 cycles of denaturation, annealing and extension at 52°C and 72°C for 60 sec respectively. Final extension was done at 72°C for 5 min and reaction is terminated by holding at 4°C. The PCR products (for genus specific PCR) were subjected to electrophoresis at 120 V/cm for 45 minutes in 2% agarose gel prepared in 0.5 x TBE buffer containing 0.5 ng/ml of ethidium bromide. The PCR products (5 µl) were run along with 100 b.p. DNA ladder (Invitrogen) and amplicons were visualized under UVP Gel Doc Bioimaging system.

Results and Discussion

Over all prevalence of enteric *Salmonella* in apparently healthy horses was found 2.72% (3/110) and 16% (8/50) in hospitalized horses on the basis of *InvA* gene specific polymerase chain reaction. In present study lower prevalence in apparently healthy horses have been recorded as compared to earlier studies (Singh *et al.*, 2007; 6.5% and Babu *et al.*, 2008; 14.5%) in India. It might be attributed to differences in management practices followed by individual owners and the fact that in present investigation almost 60% of the samples were collected from organized farms where hygienic practices were better in comparison with unorganized farms/low income group individuals in previous studies (Singh *et al.*, 2007). In present study higher prevalence of salmonella in hospitalized horses have been recorded then the previous studies carried out at abroad (Traub-Dargatz *et al.*, 1990; 7%, Ernst *et al.*, 2004; 13%), it might be due to long-distance transportation before reaching to veterinary clinic in Bikaner (Kim *et al.*, 2001; Owen *et al.*, 1983), and because a high case load at clinic of veterinary college Bikaner with mixing of variety of species in a large number may serve as source of nosocomial infection (Radostits *et al.*, 2007).

To study association of age with *salmonella* shedding horses of current study were divided into four age groups, less than 1 year of age, 1 to 4 year of age, 5 to 8 years of age and more than 8 years of age. In horses of less than 1 year of age 5% (1/20) were found positive. In horses of 1 to 4 years age 2.08% (1/48) were found positive, in horses of 5 to 8 years of age 5% (3/60) were found positive. In horses more than 8 years of age 18.75% (6/32) were positive for *Salmonella* (Table 1 and 2). The highest prevalence was observed in horses belonging to age group more than 8 years of age. The old age may play an important role in acquiring salmonella infection especially under stress and suppressed immunity (Hird *et al.*, 1986). However a few authors have not mentioned any significant relation between prevalence of enteric *Salmonella* and age of horses (Hird *et al.*, 1984; Traub-Dargatz *et al.*, 1990).

To interpret the relation of *Salmonella* positive horses with the previous history of different clinical signs data of all horses (healthy and hospitalized) were jointly assessed, because maximum numbers of horses which had showed clinical signs were belonging to the hospitalized horses. On the basis of history of antibiotic use in last 3 months horses were divided into two groups antibiotic used and antibiotic not used. In antibiotic used horses 36.00% (9/25) were found positive and in horses with no history of antibiotic use only 2.35% (2/85) were found positive (Table 1 and 2). In agreement to the present observation, association between antimicrobial drug treatment and *Salmonella*

infections has been identified in previous studies (Baker and Leyland 1973, Hird *et al.*, 1986, Smith *et al.*, 1978). The normal intestinal flora is considered an important line of defence against colonization by pathogenic bacteria. The association of exposure to antimicrobial drugs and *Salmonella* shedding can be explained by the selective elimination of intestinal normal flora antagonistic to *Salmonella* (Hird *et al.*, 1984; House *et al.*, 1999). Antibiotics provide a favourable intestinal environment for pathogenic organisms to infect host cells and decreases the number of organisms required to produce infection.

In the horses having previous history of diarrhoea in last three months 18.18% (6/33) were found positive for *Salmonella* and in the horses which did not have history of diarrhoea 6.49% (5/77) were positive for *Salmonella* shedding. In the horses having previous history of colic in last 3 months 11.76% (4/34) were found positive and in horses which did not have history of colic 9.21% (7/76) were positive for *Salmonella* shedding. Diarrhoea and colic are important clinical signs of enteric *Salmonella* infection (Ernst *et al.*, 2004; Alinovi *et al.*, 2003; Sharma and Soni, 2008). In the horses having previous history of laminitis 29.41% (5/17) were found *Salmonella* positive and in the horses which did not have history of laminitis only 6.45% (6/93) were positive for *Salmonella* shedding. In salmonellosis there is disruption of the intestinal mucosa, as the mucosa is damaged, endotoxemia commonly occurs and leads to laminitis (Krueger *et al.*, 1986). Horses with locomotor disorders remain at higher risk for salmonella infection (Ravary *et al.*, 1998). Half (3/6) of the horses which were transported from distant places to the veterinary clinic were found positive for the *Salmonella* shedding while remaining 44 horses which were belonging to local area only 11.36% (5/44) were found positive for *Salmonella* (Table 2). Transportation impinges stress and plays a major role in reactivating the *Salmonella* (Owen *et al.*, 1983). Findings of the present investigation were also in agreement with Kim *et al.*, (2001) and Alinovi *et al.*, (2003).

On the basis of antibiotics used on first day of hospitalization horses were divided into two groups antibiotics used and not used. In the group antibiotic used 18.18% (8/44) were found positive while in no antibiotic group of 6 animals none of the horse was found positive. The normal intestinal flora is considered an important line of defence against colonization by pathogenic bacteria. Association of exposure to antimicrobial drugs and *Salmonella* shedding in animals is previously explained in many studies (Hird *et al.*, 1984; House *et al.*, 1999).

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Table 1: Cross-classification of enteric *Salmonella*-PCR status by host and management factors for 110 apparently healthy horses.

| S. No. | Variable | Category | No. of positive | No. of negative |
|--------|----------------------|------------------------------|-----------------|-----------------|
| 1. | Gender | Male | 3 | 54 |
| | | Female | 0 | 53 |
| 2. | Age | <1 | 1 | 17 |
| | | 1-4 | 0 | 35 |
| | | 5-8 | 0 | 38 |
| | | >8 | 2 | 17 |
| 3. | Farming practices | Organized | 1 | 63 |
| | | Unorganized | 2 | 44 |
| 4. | Feeding practices | Availability of green fodder | 0 | 60 |
| | | Non availability | 2 | 44 |
| | | Foal on milk feeding | 1 | 3 |
| 5. | Use of horses | Breeding | 2 | 49 |
| | | Equestrian sports | 0 | 13 |
| | | Carting | 0 | 40 |
| | | Others | 1 | 5 |
| 6. | Housing | Open/loose | 1 | 56 |
| | | Stable/stall | 2 | 51 |
| 7. | No of horses at farm | 0-5 | 0 | 12 |
| | | 6-15 | 1 | 40 |
| | | >15 | 2 | 55 |
| 8. | History | Previous use of antibiotics | 2 | 7 |
| | | No use of antibiotic | 1 | 100 |
| | | Previous diarrhoea | 1 | 9 |
| | | No diarrhoea | 2 | 98 |
| | | Previous colic | 0 | 14 |
| | | No colic | 3 | 93 |
| | | Previous laminitis | 2 | 8 |
| | | No laminitis | 1 | 99 |

Table 2: Cross-classification of enteric *Salmonella*-PCR status by host and management factors for 50 hospitalized horses.

| S. No. | Variable | Category | No. of positive | No. of negative |
|--------|--------------------------------|--|-----------------|-----------------|
| 1. | Gender | Male | 3 | 16 |
| | | Female | 5 | 26 |
| 2. | Age | <1 | 0 | 2 |
| | | 1-4 | 1 | 12 |
| | | 5-8 | 3 | 19 |
| | | >8 | 4 | 9 |
| 3. | Transportation | Non-transported | 5 | 39 |
| | | Transported | 3 | 3 |
| 4. | History | Previous antibiotic use | 7 | 9 |
| | | No antibiotic use | 1 | 33 |
| | | Previous diarrhoea | 5 | 18 |
| | | No diarrhoea | 3 | 24 |
| | | Previous colic | 4 | 16 |
| | | No colic | 4 | 26 |
| | | Previous laminitis | 3 | 4 |
| | | No laminitis | 5 | 38 |
| 5. | Factors during hospitalization | Use of parenteral antibiotics (first day of hospitalization) | 8 | 36 |
| | | No antibiotic use | 0 | 6 |

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