Veterinary Integrative Sciences 2022; 20(2): 497-496 DOI; 10.12982/VIS.2022.037

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Epidemiology and antimicrobial resistance of salmonella isolated from racehorses and horsemen in Northeastern Thailand

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Abstract

Salmonella is one of the major causes of foodborne diseases in humans. These bacteria can colonize within the gastrointestinal tracts of both humans and animals, and there have been reports of incidences of *Salmonella* in horses. This study aimed to investigate the prevalence, serotypes, and antimicrobial resistance of *Salmonella* isolates from racehorses and horsemen, as well as to explore the possible transmission between horses and humans. Fecal samples from racehorses (247 samples) and horsemen (33 samples) were collected from horse farms located in 3 provinces of upper Northeastern Thailand between March and August 2019. *Salmonella* was isolated and identified. Broth microdilution was used to determine the minimal inhibitory concentrations (MICs) of the antimicrobial agents for antimicrobial. *Salmonella* isolates were detected in 4.86% (12/247) of racehorses and 3.03% (1/33) of horsemen. The most commonly found serotypes in the isolates obtained from the racehorses were Abony (25%) and Iganda (16.67%). Only the Tumodi II serotype was found in one horseman. *Salmonella* isolates collected from the racehorses had been the most resistant to streptomycin (66.67%), while the isolate from a horsemen had been resistant to ampicillin, streptomycin, oxytetracycline, and to tetracycline. Although *Salmonella* transmission between racehorses and horsemen was not found to be obviously present in this study, the appropriate use of antimicrobials and hygienic procedures are still necessary in order to prevent antimicrobial resistance and the transmission of drug-resistant *Salmonella* between horses and humans.

Keywords: Antimicrobial resistance, Horsemen, Prevalence, Racehorses, *Salmonella*

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Funding; This study was supported by the Faculty of Veterinary Medicine of Khon Kaen University in Thailand.

Article history; *received manuscript: 28 January 2022,*
revised manuscript: 19 March 2022,
accepted manuscript: : 9 June 2022,
published online: 13 June 2022
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Salmonella is a bacterium that can colonize within the gastrointestinal tracts of humans and animals. The usual symptoms, which are found in infected humans, are diarrhea, fever, and stomach cramps (CDC, 2019). Infections in animals result in various symptoms, including no evidence of symptoms, gastrointestinal tract disorders, and septicemia (Jay-Russell et al., 2014). Healthy mature horses, which are infected with *Salmonella*, are usually asymptomatic, and the infected horses become the carriers. In foals, the infection, which is usually life-threatening, causes gastrointestinal tract disorders, fevers, and septicemia at higher rates than adults (Anzai et al., 2003; Hernandez et al., 2014). The economic burdens arising from *Salmonella* infections in horses are incurred through treatment costs, recovery periods, pared-down performance, and mortality (Hernandez et al., 2014).

Infections in humans are an important public health problem. The clinical signs are usually cramps, diarrhea, nausea, vomiting, and in the case of severe infections, septicemia (CDC, 2019). Food contamination and several days of survival in the environment play important roles in the transmission of bacteria (Finn et al., 2013). Most infections are commonly self-limiting, but a severe illness or systemic infection may predispose the use of antimicrobials. In particular, antimicrobial resistant infections can also lead to complications arising from illness, including a higher hospitalization rate, a prolonged treatment time, and an increased rate of treatment failure (Varma et al., 2005; Brown et al., 2017). Antimicrobial resistance has been increasing each year, and resistance to recent generations of antimicrobials has been reported (CDC, 2019). Presently, antimicrobial resistance is a major threat to public health because it reduces antimicrobial choices for the treatment of bacterial infections, causes prolonged treatment periods, and reduces the chance of operatives that are at risk of infection. Apart from inappropriate antimicrobial use in humans, the use of antimicrobials as feed additives in livestock is the underlying source of antimicrobial resistant bacteria, which could contaminate meat and meat products and which could then be transmitted to consumers (EFSA, 2011).

In Thailand, most of the studies on *Salmonella* have been carried out on humans and food products (Bangtrakulnonth et al., 2004; Angkititrakul et al., 2005; Vaeteewootacharn et al., 2005; Yang et al., 2018). The reports showed high resistance rates of *Salmonella* to ampicillin (30% - 50%) (Suchatlikitwong and Pancharoen, 2019; NARST, 2020), but low resistance rates to ciprofloxacin (1 - 3%) (NARST, 2020) compared to reports from the CDC (2019).

Previously, most studies about *Salmonella* shedding in horses had only been carried out with hospitalized horses (House et al., 1999; Alinovi et al., 2003b). In order to assess the risk of *Salmonella* infection and transmission between the racehorses and their horsemen, the objects of this study were to examine the prevalence, serotypes, and antimicrobial resistance of *Salmonella* isolates from racehorses and horsemen, and to explore the possible transmission between horses and humans. In addition, the study of *Salmonella* in Thailand's horse population has not yet been reported.

MATERIALS AND METHODS

The Collection of Samples

From March to August 2019, fecal samples were collected from 247 healthy racehorses and 33 healthy horsemen from 30 horse racing stables. These stables were located in 3 provinces in the Northeastern region of Thailand: Nakhon Ratchasima, Khon Kaen, and Udon Thani. Fecal specimens were collected from all racehorses and horsemen by using the technique described by Janiyayotin et al., (1999). The selection criterion established for the horsemen, who were to participate in this study, was that they be individuals, who were working in close contact with the enrolled racehorses. From the racehorses, 247 fecal samples were obtained, while 33 fecal samples were obtained from the horsemen. Information, which was collected on the antimicrobials being used for the horses at that time, was acquired by directly interviewing the horsemen.

The study was approved by the Institution Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-71/62, 22/08/2019) and by the Center for Ethics in Human Research of Khon Kaen University (HE622238, 05/02/2020).

Bacterial isolation and identification

Both the isolation and identification of *Salmonella* were performed following ISO 6579-1:2017 (ISO, 2017). Briefly, ten grams of feces from the racehorses and one gram of feces from the horsemen were enriched with buffer peptone water (BPW; TM MEDIA, Rajasthan, India) at 37˚C for 24 hours. The 3 loopfuls of culture suspension were cultured onto Modified Semisolid Rappaport-Vassiliadis medium (MSRV; HIMEDIA, Mumbai, India), and were incubated at 42˚C for 24 hours. The presumptive colonies were streaked onto xylose lysine deoxycholate agar (XLD; Darmstadt, Germany), and then the culture plates were incubated at 37˚C for 24 hours. The presumptive colonies were further identified using triple sugar iron agar (TSI; HIMEDIA) and motility indole-lysine agar (MIL; HIMEDIA). *Salmonella* spp. was serotyped by the Center for Antimicrobial Resistance Monitoring in Foodborne Pathogens at Chulalongkorn University.

Antimicrobial susceptibility test

The antimicrobial susceptibility was tested by using the broth microdilution technique according to ISO 20776-1:2019 (ISO, 2019) and the Laboratory Standards Institute M100 27th standard (CLSI, 2017). Nine antimicrobials, including amoxicillin/clavulanic (AMC), ampicillin (AMP), enrofloxacin (ENR), gentamicin (GEN), oxytetracycline (OTC), polymyxin B (PMB), streptomycin (STR), tetracycline (TET), and trimethoprim (TMP), were chosen for antimicrobial susceptibility testing. This was based upon the common antimicrobials being used in racehorse stables at the time of this study and the CLSI recommendations (CLSI, 2017).

RESULTS

The prevalence of *Salmonella*, which was isolated from the racehorses and the horsemen, were at 4.86% (12/247) and 3.03% (1/33), respectively (Table 1). Abony and Inganda were the most common serotypes of *Salmonella* isolated from racehorses, and these were found at 25% (3/12) and 16.67% (2/12), respectively. The *Salmonella* isolate from the horseman belonged to serotype: Tumodi II (Table 2).

Based on the interviews with the horsemen, it was discovered that gentamicin (GEN), oxytetracycline (OTC), penicillin G, streptomycin (STR), and trimethoprim/sulfamethoxazole (SXT) had been the common antimicrobials being used in most racehorse stables.

Antimicrobial susceptibility testing revealed that the *Salmonella* isolates from racehorses had shown the highest resistance to STR (66.67%) and that all isolates had been susceptible to GEN, PMB, and TMP (Table 3). The rate of resistance to other tested antimicrobials had been at 16.67% (Table 3). The isolate from the single horseman had displayed resistance to AMP, OTC, STR, and TET (Table 4). Three out of 13 *Salmonella* isolates were found to be multidrug resistant (MDR) (Table 5). Two of these isolates were recovered from racehorses, while another isolate was recovered from a horseman. The *Salmonella* serotype, Derby, was found to be the most highly resistant isolate by resisting 6 out of 9 antimicrobials (Table 3).

Table 1 The prevalence of *Salmonella* isolates from the racehorses and the horsemen

Sources	Stables	Racehorses		Horsemen	
	(n)	Samples (n)	Positives (n, %)	Samples (n)	Positives (n, %)
Nakhon Ratchasima	14	150	5, 3.33	27	1, 3.7
Khon Kaen	5	54	4, 7.41	4	0, 0.00
Udon Thani	11	43	3, 6.98	2	0, 0.00
Totals	30	247	12, 4.86	33	1, 3.03

Table 2 Serotypes of 12 *Salmonella* isolates from the racehorses and 1 isolate from a horseman

Salmonella Groups	*Salmonella* Serotypes	Racehorses (n, %)	Horseman (n, %)
B	*S.* Abony	3, 25.00	-
	S. Stanley	1, 8.33	-
	S. Kubacha	1, 8.33	-
	S. Derby	1, 8.33	-
	S. Tumodi II	-	1, 100
C	*S.* Inganda	2, 16.67	-
	S. Eingedi	1, 8.33	-
	S. Planckendael	1, 8.33	-
E	*S.* Weybridge	1, 8.33	-
	S. Lexington	1, 8.33	-

Table 3 Serotypes of 12 *Salmonella* isolates from the racehorses and 1 isolate from a horseman

Salmonella isolations	MIC values (µg/ml)								
	AMC	AMP	ENR	GEN	OTC	PMB	STR	TET	TMP
S. Abony (a)	1	4	0.25	0.5	1	1	32^R	2	0.0625
S. Abony (b)	1	4	0.25	1	1	1	16	64^R	0.25
S. Abony (c)	2	4	0.5	0.5	1	1	16	2	0.25
S. Stanley	1	4	0.5	0.5	1	2	32^R	2	0.25
S. Kubacha	1	8	0.25	0.25	64^R	1	64^R	4	0.125
S. Derby	16^R	64^R	4^R	0.5	64^R	1	32^R	64^R	0.25
S. Inganda (i)	32^R	4	0.5	0.5	1	1	32^R	2	0.0625
S. Inganda (ii)	1	4	0.5	1	1	1	16	2	0.5
S. Eingedi	1	64^R	1^R	1	1	2	64^R	2	0.5
S. Planckendael	1	16	0.25	1	1	1	64^R	1	0.5
S. Weybridge	1	4	0.5	0.25	1	1	16	2	0.5
S. Lexington	1	8	0.25	0.5	1	1	64^R	1	0.5
R/n	2/12	2/12	2/12	0/12	2/12	0/12	8/12	2/12	0/12
(%)	*(16.67)*	*(16.67)*	*(16.67)*	*(0)*	*(16.67)*	*(0)*	*(66.67)*	*(16.67)*	*(0)*

Note: MIC: Minimum inhibitory concentration, AMC: amoxicillin/clavulanic, AMP: ampicillin, ENR: enrofloxacin, GEN: gentamicin, OTC: oxytetracycline, PMB: polymyxin B, STR: streptomycin, TET: tetracycline, and TMP: trimethoprim,
(a) and (c): The samples from Khon Kaen but from different stalls.
(b) : The samples from Nakhon Ratchasima.
(i) and (ii): The samples from Udon Thani and Khon Kane subsequently.
R: resistance by superscript
R: number of resistances

Table 4 The MIC values of antibiotics towards *Salmonella* isolated from the horseman

Salmonella isolations	MIC values (µg/ml)								
	AMC	AMP	ENR	GEN	OTC	PMB	STR	TET	TMP
S. Tumodi II	8	64^R	0.25	0.5	64^R	1	64^R	64R	0.25

Note: ^{R}: resistance by superscript

Table 5 The antimicrobial resistance profiles of *Salmonella* from the racehorses and the horseman

Salmonella isolates	Racehorses	Horseman
S. Abony (a)	STR	
S. Abony *(b)*	TET	
S. Abony *(c)*	-	
S. Stanley	STR	
S. Kubacha	OTC-STR	
S. Derby	AMC-AMP-ENR -OTC -STR-TET	
S. Tumodi II	-	AMP-OTC -STR-TET
S. Inganda *(a)*	AMC-STR	
S. Inganda *(b)*	-	
S. Eingedi	AMP-ENR –STR	
S. Planckendael	STR	
S. Weybridge	-	
S. Lexington	STR	

DISCUSSION

In this study, the prevalence of fecal *Salmonella* shedding from racehorses was found to be 4.86% (12/247). The percentage of *Salmonella* shedding was higher than a report from the United States at 0.8% (Traub-Dargatz et al., 2000), in a study of endurance horses at 0.5% (Fielding et al., 2013), and in a study in racehorses in Louisiana at 2.1% (Chapman, 2006). Nonetheless, the percentage of racehorses excreting *Salmonella* in our study was lower than those studies, which had been conducted on hospitalized horses. *Salmonella* was detected at a rate of between 9 to13% in hospitalized horses (House et al., 1999; Kim et al., 2001) and was found to be as high as 26 % in horses with diarrhea (Alinovi et al., 2003a).

Many factors associated with the prevalence of *Salmonella* shedding have been described. In healthy horses, stress factors, such as transporting, shipping, withdrawing feed, and changing feed, have been associated with *Salmonella* shedding (Hernandez et al., 2014). Higher ambient temperatures could enhance the survival and multiplication of *Salmonella* and could lessen a horse's immune response (House et al., 1999). Moreover, this has been associated with the reporting of peak equine salmonellosis cases in summer (Carter et al., 1986). The predisposing factors for *Salmonella* infections consisted of the following: 1) the use of antimicrobials, especially the use of potassium penicillin G, in the hospitalized horses (House et al., 1999); 2) the withholding of feed; and 3) the changing of the feed. These three were found to alter the normal flora and to reduce the infection dose of *Salmonella* (House et al., 1999; Hernandez et al., 2014).

In this study, most of the racehorse stables were located in residential areas and had no paddocks or tracks. All the time, the horses were being transported and shipped to competitions or to training sessions at the training track. The duration of the transport period could range from 30 minutes to 6 hours per day, especially on competition days. Furthermore, due to a lack of paddocks at most of the stables, most of the racehorses would spend nearly 20 hours per day in the stalls, leaving only 3 to 4 hours per day for exercising, strolling, and grooming. These stress factors correlate with findings from a study by Hernandez et al. (2014). In addition, uncontrollable factors, such as high ambient temperatures, have been reported to be associated with increased rates of *Salmonella* shedding (Carter et al., 1986; House et al., 1999). The tropical climate in our region may have been one of the factors that contributed to *Salmonella* shedding in horses in our study.

In addition, the use of antimicrobials was a factor in this study, which correlated with results from studies by House et al. (1999) and Hernandez et al. (2014). Most racehorse stables were found to be using antimicrobials without a veterinary prescription. Penicillin G procaine and STR combinations were found to be the most commonly used antimicrobials, which was related to the high resistance rate of streptomycin (66.67%) in this study. Although susceptibility to penicillin G was not tested in this study, resistance to aminopenicillins was determined (AMC 16.67%, AMP 16.67%). Gentamicin, oxytetracycline, and trimethoprim/sulfamethoxazole were also being commonly used, but to a lesser extent than penicillin G. However, the *Salmonella* isolates were still susceptible to gentamicin and trimethoprim.

The virulent serotype of *Salmonella* in horses has not been indicated (Hernandez et al., 2014). Abony (25%, 3/12) and Inganda (16.67%, 2/12) were the two most common serotypes, which had been isolated from the racehorses in this study. However, in previous studies, these two serotypes were not present in healthy horses (McCain and Powell, 1990; Traub-Dargatz et al., 2000). In previous studies, the serotypes of Derby, Stanley, and Lexington were detected in meat, meat products and humans in the Northeastern region of Thailand (Angkititrakul et al., 2005; Vaeteewootacharn et al., 2005; Yang et al., 2018). We also found these three serotypes, but at a low percentage (8.33%). The same *Salmonella* serotype, Abony, was recovered from 2 out of 17 horses living in the same stable, which suggests that within a stable, the transmission of *Salmonella* might occur between racehorses. Generally, these racehorses were individually managed, and their direct contact with each other was limited. Therefore, transmission may have been derived from indirect contact with vehicles, such as birds, insects, equipment, and groomers.

The percentage of fecal *Salmonella* shedding from horsemen was at 3.03% (1/33). Our finding was relatively similar to a previous study, which had reported a *Salmonella* detection rate of 4.7% (19/403) in healthy humans residing in the Northeastern region (Vaeteewootacharn et al., 2005). The only isolate found in a horseman in this study belonged to the serotype of Tumodi II, which was not found to match any isolates from the racehorses. In the previous reports (Angkititrakul et al., 2005; Vaeteewootacharn et al., 2005; Yang et al., 2018), Rissen, Stanley, Virchow, Anatum, and Enteriditis were the most common serotypes, which had been isolated from humans and food chains in the Northeast. However, these serotypes were not detected in this study in either the racehorses or the horsemen. In addition, Tumodi II was not present in the national report (Bangtrakulnonth et al., 2004) that investigated the presence of *Salmonella* in human shedding and other sources.

In this study, Streptomycin was found to have the highest resistance rate among the *Salmonella* isolates, and the resistance of the isolates to this drug was present in both racehorses (66.67%) and in one horseman. The findings in this study were in accordance with a report regarding healthy humans (Sringam et al., 2016), in which *Salmonella* isolates from humans had been resistant to AMP, STR, and TET, but had been susceptible to AMC. Sringam et al. (2006) reported that ampicillin had the highest resistance rate at about 60% to 70%, which was similar to findings from other studies (Suchatlikitwong and Pancharoen, 2019; NARST, 2020). However, resistance to Streptomycin was found to vary and ranged from 11% to 45% (Sringam et al., 2016).

CONCLUSION

The *Salmonella* serotypes differed from the previous studies (Angkititrakul et al., 2005; Vaeteewootacharn et al., 2005; Yang et al., 2018). It is possible that most of these racehorses had been imported from foreign countries. To be clear, *Salmonella* identification in the original racehorses or a comparison of DNA fingerprints should be required. The most resistant antibiotic in racehorses was STR, which is associated with antimicrobial use in racehorse stables. There was no evidence of *Salmonella* transmission between

the racehorses and the horsemen, but the transfer between racehorses within a stable was found to be possible. In order to limit *Salmonella* transmission, hygienic precautions are essential, and a probiotic feed additive could also help to minimize the shedding of *Salmonella* in horses (Kim et al., 2001). Appropriate antimicrobial usage is requested to reduce the development of antibiotic resistance and the possibility of *Salmonella* transmissions, particularly drug-resistant *Salmonella*, which was reported (Karon et al., 2007).

ACKNOWLEDGEMENTS

The authors would like to thank the stakeholders of the racehorse stables in Nakhon Ratchasima, Khon Kaen, and Udon Thani for volunteering, and the Center for Antimicrobial Resistance Monitoring of Foodborne Pathogens at Chulalongkorn University for supporting the *Salmonella* serotyping.

AUTHOR CONTRIBUTIONS

Ruxpon Dejkong assisted in conducting the experiments, performed data visualization and wrote the manuscript. Sunpetch Angkititrakul, Suchat Wattanachai, Suphannika Putthachalee, and Patchara Phuektes design and conducted all the experiments and wrote the manuscript. All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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How to cite this article;

Ruxpon Dejkong, Suchat Wattanachai, Patchara Phuektes, Suphannika Putthachalee and Sunpetch Angkititrakul. Epidemiology and antimicrobial resistance of salmonella isolated from racehorses and horsemen in Northeastern Thailand. Veterinary Integrative Sciences. 2022; 20(2): 497- 506.