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Detection of *Salmonella* spp., in Delman Horses at Berastagi Fruit Market, Karo, North Sumatra, Indonesia

(DETEKSI SALMONELLA SPP., PADA KUDA DELMAN DI PASAR BUAH BERASTAGI, KARO, SUMATRA UTARA, INDONESIA)

Darniati[1 *], Audra Vinela[2], Fakhrurrazi[1]

[1]Laboratory of Veterinary Microbiology,
[2]Undergraduate Programe Study, Faculty of Veterinary Medicine,
Universitas Syiah Kuala, Jalan Tgk. Hasan Krueng Kalee 4,
Darussalam Banda Aceh, Aceh, Indonesia 23111
*Email: darni_zain@usk.ac.id

ABSTRACT

Salmonella spp., is one of the pathogenic bacteria in humans and animals. Multiple *Salmonella* serovars are a global threat to public health due to increasing prevalence and antimicrobial resistance. This study was aimed to identify the presence of *Salmonella* spp., on the feces of *delman* horses (horse-drawn carriage), with or without diarrhea symptoms at Berastagi Fruit Market, Karo, North Sumatra, Indonesia, and determine its antimicrobial resistance status. The fecal samples were collected from 10 diarrheal and five healthy horses. Samples were isolated in Selenite Cystine broth and Salmonella-Shigella agar, and identification was done through biochemical tests. The results showed that *Salmonella* spp., can be found in 90% (n=10) diarrheal horses and 100% (n=5) in healthy horses. The species were identified as *Salmonella typhi* (13%), *S. enteritidis* (13%), *S. arizonae* (27%) and *S. typhimurium* (40%). *Salmonella typhimurium* showed the highest resistance rate to erythromycin and tetracycline. *Salmonella enteritidis* and *S. typhi* demonstrated the highest resistance to tetracycline and erythromycin. *Salmonella arizonae* showed 100% resistance to erythromycin. In conclusion, *Salmonella* spp., can be isolated from healthy and diarrhea *delman* horses and showed potential antimicrobial resistance.

Keywords: *Salmonella* spp.; Horse; Diarrhea; Public health

ABSTRAK

Salmonella spp., merupakan salah satu bakteri patogen bagi manusia dan hewan. Berbagai serovar *Salmonella* merupakan ancaman global bagi kesehatan masyarakat karena prevalensi dan resistansi antimikrob yang semakin meningkat. Penelitian ini bertujuan untuk mengidentifikasi *Salmonella* spp., pada tinja kuda delman dengan atau tanpa gejala diare di Pasar Buah Berastagi, Karo, Sumatra Utara, Indonesia dan menguji kepekaannya terhadap antibiotik. Sampel feses dikumpulkan dari 10 ekor kuda yang mengalami diare dan lima ekor kuda yang sehat. Sampel diisolasi menggunakan media *Selenite Cystine Broth* dan

Salmonella-Shigella agar, dan identifikasi dilakukan melalui uji biokimia sederhana. Hasil penelitian menunjukkan bahwa *Salmonella* spp., dapat ditemukan pada 90% (n =10) kuda diare dan 100% (n =5) kuda sehat. Spesies yang diidentifikasi adalah as *Salmonella typhi* (13%), *S. enteritidis* (13%), *S. arizonae* (27%), dan *S. typhimurium* (40%). *Salmonella typhimurium* menunjukkan tingkat resistansi tertinggi terhadap eritromisin dan tetrasiklin. *Salmonella enteritidis* dan *S. typhi* menunjukkan resistansi tertinggi terhadap tetrasiklin, eritromisin. *Salmonella arizonae* menunjukkan resistansi 100% terhadap eritromisin. Berdasarkan hasil penelitian dapat disimpulkan bahwa *Salmonella* spp., dapat diisolasi dari kuda delman yang sehat dan diare serta menunjukkan potensi resistansi antimikrob.

Kata-kata kunci: *Salmonella* spp.; kuda; diare; kesehatan masyarakat

INTRODUCTION

Horses (*Equus caballus* or *Equus ferus caballus*) are one of the large livestock that play important roles in people's community. Horses are utilized by society as pets, for sports, recreational facilities and as a means of transportation (Gaina and Foeh, 2018). In Indonesia, horses are also used for meat and milk production, as racehorses and to pull carriages such as *andong* or *delman* (horse-drawn carriage) (Apriliawati *et al*., 2019). *Delman* is a form of traditional transportation that uses horses as propulsion and is often used to attract - tourists in various regions, including Berastagi Fruit Market, Karo, North Sumatra (Suranny, 2016).

Currently, the population has declined due to often resulting from factors, including environmental pressures and infectious diseases (Ariman *et al*., 2021). *Delman* horses are among the populations in a concerning condition. On average, *delman* horses are also reported to be susceptible to digestive disorders such as gastrointestinal parasites, bacterial infections and colic (Purnama *et al*., 2022). Excessive activity and poor environmental conditions make horses more susceptible to various infectious agents including parasites, fungi, viruses, and bacteria (Sari *et al*., 2018). Some of the pathogenic bacteria associated with colitis include *Clostridium perfringens*, *C. difficile*, *Neorickettsia risticii* and *Salmonella* spp. (Garber *et al*., 2020).

Salmonella spp., is a rod-shaped, Gram-negative bacteria belonging to the Enterobacteriaceae family (Kalambhe *et al*., 2016; Marks *et al*., 2017). The genus *Sal-monella* is divided into two species, *Salmonella bongori* and *S. enterica*. *Salmonella enterica* is further subdivided into six subspecies; subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. Furthermore, *S. enterica* is differentiated into over 2,600 distinct serovars based on their various biochemical characteristic (Quiñones *et al*., 2024).

Salmonella spp. can infect a wide variety of animal species, including mammals, fish and birds. The main modes of infection are fecal-oral (Worley 2023), inhalation (Pal *et al.*, 2021) and transplacental (Betancourt *et al*., 2021). Clinical symptoms generally appear 4-72 hours after infection (Rawat *et al*., 2023). The symptoms caused by *Salmonella* spp., typically include diarrhea, fever, vomiting, abdominal pain and septicemia leading to multiple organ failure (Aoki *et al*., 2017). Infections may present asymptomatic to manifest as inflammatory, diarrhea or typhoid fever depending on serovar and host-specific factors.

In horses, the most common symptoms of salmonellosis include diarrhea, fever, colic and leukopenia (Majhut *et al*., 2019). *Salmonella* spp., infection in adult horses mostly associated with enterocolitis, causing diarrhea and hypoproteinemia. Atypical presentations of salmonellosis in horse include gastrointestinal gastric reflux (Rothers *et al*, 2020), with or without clinical signs (Burgess, 2023). Horses with subclinical infection act as carriers, shed-ding

Salmonella spp., into the environment without showing clinical signs, thereby posing a potential risk to fecal contamination disseminating throughout the facility (Burgess *et al*., 2023).

Foodborne pathogens such as *Salmonella* spp., are difficult to control due to their ability to survive in the environment (Liu *et al*., 2018). *Salmonella* spp., contamination in food products is a major concern for community health due to the potential for humans infection through consumption of contaminated food. Thus, *Salmonella* spp., infection remains a significant global issue (Popa *et al*., 2021). Infection by *Salmonella* spp., results in economic losses and negatively impact public health (Yada *et al*., 2023). Therefore, it is important to understand the ecology of *Salmonella* spp., in animals, and the risk of environment contamination, particularly through human activities such as those occurring in markets.

Since these bacteria can survive outside of their natural habitat, fresh products like fruits and vegetables can be contaminated by the feces of infected animals. Furthermore, early detection of microorganisms in infected animals plays an important role in preventing outbreaks. Therefore, this study was conducted to identify Salmonella species in the horse population at market locations, aiming to play an important role in preventing foodborne outbreaks.

RESEARCH METHODS

Specimens Collection

Stool specimens were aseptically collected via rectal swabs from 15 horses, including 10 diarrheal and five healthy horses, at Berastagi Fruit market, Karo, North Sumatera. All samples were placed in sterile plastic bags, transported to the Laboratory under a cold chain and stored at -20°C until examined.

Bacteria Cultivation

For pre-enrichment, each of stool specimens were transferred into tubes containing 10 mL Selenite Cystine Broth/SCB (CM0699B Selenite Cystine Broth Base®, Oxoid, Basingstoke, Hampshire, United Kingdom) and incubated at 37°C for 18 hours. The growth of *Salmonella* spp., on SCB media marked by a change in the color of the media to pink-orange-red. Subsequently, one loopful of positive sample cultured was streaked onto Salmonella-Shigella agar (Thermo Scientific Oxoid SS Agar®, Oxoid, Basingstoke, Hampshire, United Kingdom) and incubated at 37°C for 24 hours. The plates were examined for the presence of typical colonies of *Salmonella* spp.

Morphological Characterization

Bacterial characterization was performed through Gram’s staining. One loopful of an isolate from the representative Salmonella colonies was picked from Salmonella-Shigella agar, homogenized with natrium chloride (NaCl) on an object glass and fixed by gentle heating. Crystal violet was applied to the slide for two minutes, followed by rinsing with distilled water. Gram’s iodine was added for one minute, after which acetone alcohol was used for a few seconds. Finally, safranin was applied for two minutes, the slide was rinsed again with distilled water, air-dried and observed under a microscope 1000 times magnifycation.

Biochemical characterization

Isolated organisms exhibiting Salmonella characteristics were subjected to biochemical tests, including Triple Sugar Iron Agar, citrate utilization, Methyl Red-Voges Proskauer (MR-VP), indole production, Sulfid indole motility test and sugar fermentation test (glucose, sucrose, maltose, mannitol and Lactose).

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was conducted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). The Kirby-Bauer disk diffusion technique was applied using antibiotic discs containing, ciprofloxacin (5 μg), tetracycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), imepenem (10 μg) and chloramphenicol (30 μg). Bacterial suspensions were standardized to 0.5 McFarland turbidity (equivalent to 1.5×10^8 CFU/mL), and evenly spread onto Mueller-Hinton Agar plates (Mueller Hinton Agar (MHA), CM0337B®, Oxoid, Basingstoke, Hampshire, United Kingdom). Inhibition zones were measured and interpreted as susceptible, intermediate, or resistant.

Data Analysis

The results were analyzed descripttively by observing the presence of *Salmonella* spp., in the positive sample.

RESULTS AND DISCUSSION

Based on the ability to grow in Selenite Cystine Broth (SCB), diarrheic stool samples showed positive for Salmonella growth up to 90% (9/10). Meanwhile, non-diarrhea stool showed positive results up to 100% (Table 1). Basically, sodium selenite is toxic in low concentration to most bacteria, including *Salmonella* spp. However, the ability of *S*almonellas to grow in selenite broth due to selenite binding by peptone constituents. Reduction of selenite takes place after growth is established and the intensity of reduction is related to the profuseness of growth (Chen *et al*., 1994). In a Salmonella isolation procedure using SCB, a positive sample is indicated by a color change to orange. The color change of the SCB media to orange is caused by the sodium selenite inhibitor content being reduced to elemental selenium (Eswayah *et al*., 2016).

The presence of *Salmonella* spp., in SCB medium can be confirmed by inoculating the sample on Salmonella-Shigella Agar media. *Salmonella* spp., cultured in SS agar medium was conducted on 14 samples that showed positive results in the SCB media. This result is in accordance with the interpretation of Salmonella growth on SCB media. All Salmonella isolated produced black colonies on SS agar. *Salmonella* spp., typically produces hydrogen sulfide (H_2S), which exhibit in the formation of black colonies or black centers (Yanestria *et al*., 2019). The bacterial morphology showed round, convex, shiny surfaces and *smooth* colonies. According to the cell wall structure and chemical composition, *Salmonella* spp., grouped into Gram-negative and rod-shaped bacteria (Bano *et al*., 2020). This study showed pink, rod-shaped bacteria in Gram staining as seen in Figure 3.

The most infection in humans reported cause by *S. typhi* and *S. paratyphi* and causes of disability and death (Stanaway *et al.*, 2019). Based on the biochemical tests, it can be seen that the prevalence of *Salmonella* spp. in *delman* horse feces at Berastagi Fruit Market reached 93% (Table 2). The delman horse infected by several species of *Salmonella* spp., including *S. typhi* (13%, 2/15), *S. enteritidis* (13%, 2/15), *S. arizonae* (27%, 4 /15) and *S. typhimurium* (40%, 6/15).

Salmonella typhi is a pathogenic-bacteria that causes typhoid fever, also known as typhus. Typhoid fever is a serious illness that can spread through contaminated food or water and can lead to complications if left untreated (Brockett *et al*., 2020; Marchello *et al*., 2020).

Salmonella typhi is highly resistant to selenite and sodium deoxycholate and capable to eliminate the enteric bacteria, producing endotoxins, and CFA/I as a crucial role in Salmonella attachment and mannose-resistanthemagglutination (MRHA) (Olivar-Casique *et al*., 2022; Yang *et al*., 2018). Infected host can shed the pathogen through feces for more than a year (Muturi *et al*., 2024). This pathogen is a facultative intracellular parasite, live in macrophages and cause gastrointestinal symptoms (Bula-Rudas *et al*., 2015; Harrel *et al*., 2021).

Salmonella typhimurium and *S. enteritidis* are the most frequently reported pathogens as causes of enteritis. Both of these serotypes are reported as the main important pathogens that are frequently transmitted from animals to humans around the world (López-Martín *et al*., 2016). *Salmonella* spp., infection typically localizes to the ileum, colon and mesenteric

Tabel 1. Interpretation of *Salmonella* spp. growth on selenite cystine broth media

Sampel	Positive sample	Percentage
Diarrhea stool	9	90%
Non-Diarrhea stool	5	100%

Table 2. Identification Salmonella species based on biochemical properties

Indol	MR	VP	TSIA Slant/Butt	SIM	SCA	Sucrose	Glucose	Maltose	Mannitol	Lactose	Species
-	+	-	R/Y +H2S	+	+	-	+	+	+	+	*Salmonella arizonae*
-	+	-	R/Y +H2S	+	+	-	+	+	+	-	*Salmonella enteritidis*
-	+	-	R/Y +H2S	+	-	-	+	+	+	-	*Salmonella typhi*
+	+	-	Y/Y	+	-	-	+	+	+	+	-
-	+	-	R/Y +H2S	+	+	+	+	+	+	+	*Salmonella typhimurium*
-	+	-	R/Y +H2S	+	+	+	+	+	+	+	*Salmonella typhimurium*
-	+	-	R/Y +H2S		+	+	+	+	+	+	*Salmonella typhimurium*
-	+	-	R/Y +H2S		+	+	+	+	+	+	*Salmonella typhimurium*
-	+	-	R/Y +H2S		-	-	+	+	+	-	*Salmonella typhi*
-	+	-	R/Y +H2S		+	+	+	+	+	+	*Salmonella typhimurium*
-	+	-	R/Y +H2S		+	-	+	+	+	+	*Salmonella arizonae*
-	+	-	R/Y +H2S		+	-	+	+	+	+	*Salmonella arizonae*
-	+	-	R/Y +H2S		+	-	+	+	+	-	*Salmonella enteritidis*
-	+	-	R/Y +H2S		+	-	+	+	+	+	*Salmonella arizonae*
-	+	-	R/Y +H2S		+	-	+	+	+	+	*Salmonella typhimurium*

Note: R= Red; Y= yellow

Table 3. Prevalence of antimicrobial resistance percentage in *Salmonella* spp.

Antibiotics	Number of isolates tested							
	S. typhi		*S. enteritidis*		*S. arizonae*		*S. typhimurium*	
	Number of isolates	% of resistance	Number of isolates	% of resistance	Number of isolates	% of resistance	Number of isolates	% of resistance
Tetracycline	2	100	2	100	4	25	6	100
Ciprofloxacin	2	0	2	0	4	0	6	33
Chloramphenicol	2	50	2	0	4	0	6	16
Erythromycin	2	100	2	100	4	100	6	100
Imipenem	2	50	2	0	4	0	6	16
Gentamicin	2	100	2	50	4	25	6	66

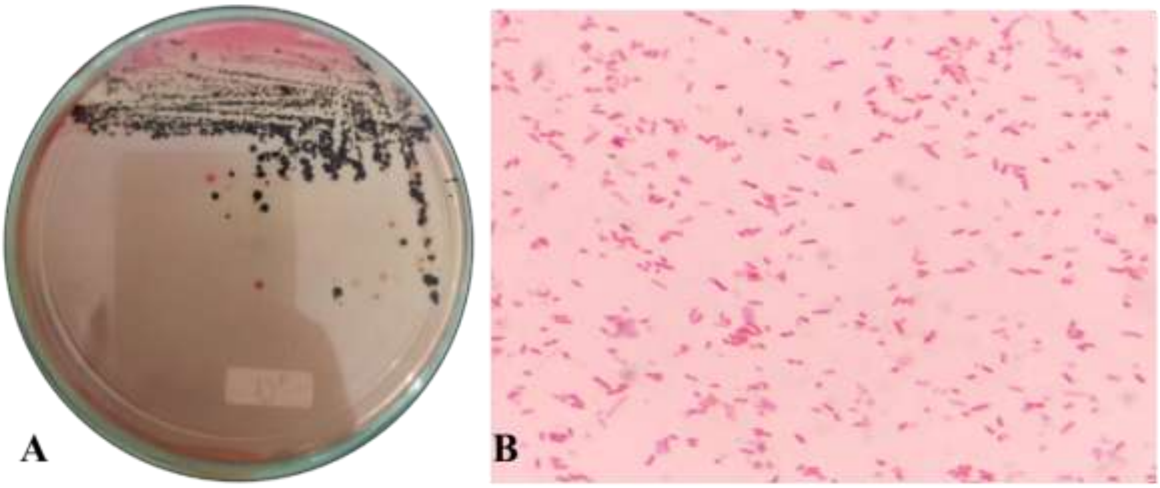

Figure 1. Salmonella morphology. A. Salmonella colony from horses stool in Salmonella-Shigella Agar. B. Rod-shape negative Gram bacteria associated with Salmonella spp.

omphalitis, hepatitis, splenitis, peritonitis and meningoencephalitis (Shivaprasad *et al*., 2006; Di Bella *et al*., 2011). *Salmonella arizonae* is an environmental pathogenic bacterium that infects various vital organs and arise serious illness in humans and ani-mals, including horses (Ahmed *et al*., 2020). *Salmonella arizona* reported causes abortion in mares due to metritis and septic placentitis resulting in fetal septicemia (May-hew *et al*., 2021)

Salmonella spp., generally had a nonspecific host. With the result that, this pathogen can infect various types of animals and humans. The spread of these bacteria between human and animals, occur directly through contact with faeces or indirectly through contaminated food or feed and drinking water. Wide distribution of agents in the environment resulting in persistence and difficult to eradicate (Zelpina and Noor, 2020). Therefore, the application of biosecurity with cage sanitation, equipment and the environment are very important to prevent the spread of infection between animals and transmission to humans.

Currently, increased antimicrobial resistance and the exhaustion of therapeutic options are major public health concerns. Cuypers *et al*. (2018) and Van Puyvelde *et al*. (2018) stated that, clinical strains of *Salmonella* spp., are usually resistant to first-line antibiotics and the antibiotics of choice for the treatment of invasive Salmonellosis in humans. Multi-drug resistance (MDR) Salmonella is a major problem in equine infections due to high mortality and financial cost of treatment (Burgess *et al*., 2018). In our study, the antibiotic susceptibility test results reveal varying resistance profiles among different Salmonella species isolated from horses (Table 3). Overall, high resistance levels were observed across several antibiotics, indicating potential antimicrobial resistance (AMR) threats. *Salmonella typhimurium*, the most frequently isolated species, showed the highest resistance rate to erythromycin (100%), tetracycline (100%) and gentamicin (66%). This is consistent with previous studies that reported the emergence of multidrug-resistant (MDR) *S. typhimurium* strains in both humans and animals (Ranjbar *et al*., 2019). Paula *et al.* (2020) reported, nine isolates of *S. typhimurium* isolated from horses displayed resistance amoxicillin/ clavulanate, ampicillin, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, trimethoprim/sulfamethoxazole and tetracycline. The isolates obtained in our study were resistant to fluoroquinolones, β-lactams, aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole and amphenicol antibiotics.

Salmonella enteritidis and *S. typhi* also demonstrated high resistance to gentamicin, tetracycline and erythromycin, suggesting widespread resistance among these serovars. This finding corroborates the concerns over the unwise use of antibiotics, which can promote the transmission of resistance genes (Van Boeckel *et al*., 2019).

In the research, *S. arizonae* displayed 100% resistance to erythromycin and sensitive to cipfofloxaxin, chloramphenicol and imepenem. Lebdah *et al.* (2017) reported that, Salmonella isolates were susceptible to ciprofloxacin (76.2%); norofloxacin (71.2%); cefotaxime (52.4%) and amikacin (80.9%). Although historically considered less pathogenic, this species' resistance profile warrants attention due to its zoonotic potential (Rabsch *et al*., 2001; Lee *et al*., 2016). The widespread resistance to erythromycin and tetracycline observed in almost all isolates may reflect the common use of these antibiotics in livestock treatment. Tetra-cyclines are among the most used antibiotics in food-producing animals globally, contri-buting to the selection pressure that fosters resistance (Chopra and Roberts, 2001).

The findings from this study highlight the urgent need for antimicrobial stewardship in animal husbandry, particularly in working animals like horses that are in frequent contact with humans. Routine monitoring and restrictions on non-therapeutic antibiotic use are critical to mitigate the spread of resistant Salmonella strains and reduce zoonotic transmission risk.

CONCLUSION

The prevalence of Salmonella was 99% in sick horses and 100% in healthy horses. *Delman* horses at the Berastagi fruit market were infected with several Salmonella serovars, including *S. typhi*, *S. typhimurium*, *S. enteritidis* and *S. arizonae*, all shoowing resistance to various antibiotics. The presence of potentially pathogenic Salmonella at the market poses a significant public health concern. Periodic studies are needed to evaluate the status of these zoonotic pathogens in food products to protect both animal and public health.

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