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A Bacteriological Study of Enterocolitis Outbreak in Horses

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Abstract

From July 2022 to January 2023, seven police training horses died after recurrent colic and remaining 27 horses were also apparently showing lethargy and mild colic signs intermittently. The investigation revealed marked neutrophilia indicating a bacterial infection. Bacteriological analysis of rectal swabs from surviving horses yielded a total of 137 bacterial isolates belonging to 17 *species* including *Acinetobacter calcoaceticus* (6), *Aeromonas schubertii* (1), *Bacillus cereus* (4), *Budvicia aquatica* (3), *Citrobacter freundii* (1), *Edwardsiella tarda* (37), *Lelliottia amnigenus* (22), *Escherichia coli* (27), *Hafnia alvei* (3), *Pantoea agglomerans* (4), *Pectobacterium cacticida* (1), *Pectobacterium rhapontici* (1), *Pseudomonas aeruginosa* (4), *Raoultella terrigena* (3), *Salmonella enterica* ssp. *arizonae* (2), *Yersinia enterocolitica* (14), and *Yersinia kristensenii* (4) were isolated. Most of the bacteria were isolated from a few horse samples, but *E. tarda*, *L.amnigenus*, *Y. enterocolitica*, and *Y. kristensenii* from 13, 12, 6 and 3 samples, respectively. The agglutination titres in horse serum sample were very high for *Y. enterocolitica* (4646 ± 2514), *E. tarda* (3058 ± 2605), *Y. kristensenii* (2299 ± 1804) and *L. amnigenus* (2216 ± 2399) while for other bacteria either agglutination was not observed or titres were very low indicating the role of the four pathogens in causation of enterocolitis. Feeding of ajowan (*Tachyspermum ammi*) seeds to horses for a week relieved all horses' signs of colic they were showing intermittently, and after January 2023 to date no case of colic or other sickness is reported from the horses.

Keywords: *Tachyspermum ammi* (Ajowan); *Edwardsiella tarda*; *Lelliottia amnigenus*; *Yersinia enterocolitica*; *Yersinia kristensenii*; *Salmonella enterica* spp. arizonae

Introduction

Enterocolitis is an inflammation of the small intestine and colon, in horses leading to colic with poor prognosis and is a common problem, globally [1]. The most common cause of enterocolitis either haemorrhagic or necrotic in foals is *Clostridium difficile* but in adult horses other bacterial causes (*Clostridium piliforme*, *Salmonella* spp., *Rhodococcus equi*, *Ehrlichia risticii*, and *Lawsonia intracellularis*) may also be important [2,3]. Besides bacterial causes several other causes including Rota virus, Corona virus, strongyles and toxicity of Non-Steroidal

Inflammatory Drugs (NSAIDs) have also been reported to cause enterocolitis in young horses [2]. This report describes identification of mixed infection of *Yersinia enterocolitica*, *Edwardsiella tarda* and *Lelliottia amnigenus* leading to the death of seven police horses in a stable after severe colic as consequence of acute and haemorrhagic enterocolitis.

Case History

From July 2022 to January 2023, seven police training horses were admitted one after the other with apparent and recurrent colic (one each in July and September 2022, two each in November and December 2022, and one in January 2023). All died during treatment and deaths were diagnosed due to acute haemorrhagic and or necrotic enterocolitis, or rupture of caecum with haemorrhagic enterocolitis based on lesions seen during post-mortem. The remaining 27 horses in the stable were lethargic, showing mild signs of abdominal pain and stretching with reduced food intake. Puzzled with the continued deaths, seven deaths of 34 horses alarmed the authorities and a systematic investigation was requested in January 2023.

Investigation

Taking clues from post-mortem reports, it was decided to take deep rectal swabs from all surviving horses along with blood samples for differential and total leukocyte count and serum on 25th January 2023. Besides, water sample supplies from a deep bore-well (SW) and a water tank (RW) were also collected along with stable soil from two places. All the samples were brought on ice and processed for isolation of all possible bacteria including aerobic, micro-aerobic, and anaerobic using standard techniques described earlier [4,5].

Bacterial isolates were characterised using conventional morphological, growth, cultural and biochemical characteristics [4-7]. All the bacterial isolates were tested for Antimicrobial Susceptibility (AST) using the Kirby-Bauer disk diffusion susceptibility test against nine herbal and nine antibiotic disks as per CLSI guideline [8]. Herbal disks were made from pure ajowan (*Tachyspermum ammi*) oil, holy basil (*Osmium sanctum*) oil, cinnamaldehyde, carvacrol, lemongrass (*Cymbopogon citratus*) oil, thyme (*Thymus vulgaris*) oil, citral, cinnamon (*Cinnamomum verum*) oil, sandalwood (*Santalum album*) oil purchased from Sigma Aldrich as per protocol detailed earlier to contain 1mg of the active ingredient per disk [9]. All antibiotic disks including amikacin (30 µg), ceftriaxone (30 µg), ceftriaxone + tazobactam (30 + 10 µg), chloramphenicol (25 µg), ciprofloxacin (10 µg), imipenem (10 µg), minocycline (30 µg), nitrofurantoin (300 µg), and tetracycline (30 µg), were procured from Difco BBL. The diameters of zones of inhibition around the herbal and antibiotic disks were measured in millimetre (mm) and interpreted for determining susceptibility or resistance as per CLSI guidelines [8] for antibiotics, and any measurable zone of inhibition around herbal disks was taken as indicator of susceptibility of the bacteria for the herbal antimicrobial [9]. For detection of Extended Spectrum β-Lactamase (ESBL) production ability of the bacterial isolates, an E-strip assay was performed as per directions of CLSI [8] using E-strips (CT/CTL 16/1, Tz/TZL 32/4, and PM/PML 16/1) from bioMerieux India and results were interpreted as per the guidelines of the manufacturer.

The antimicrobial susceptibility data were entered in an Excel sheet and analysed to determine various Antimicrobial Susceptibility Types (ASTs) and their relatedness using standard statistical analysis.

Result

During necropsy examination of the dead horses, grossly, congestion was seen in the serosa of the distal part of the small intestine, caecum and colon. Intestinal wall was thickened, oedematous, occasionally with few to multiple nodules. Mucosa was thickened, oedematous, congested and haemorrhagic. Denudation of the mucosal layer was seen occasionally with mucus or blood mixed intestinal content. Microscopically, mucosa revealed denudation of the enterocytes, haemorrhages and infiltration of the mononuclear cells and macrophages in the lamina propria as well as in the submucosal region. There was shortening of the villi, with denudation and necrosis of enterocytes and blood capillaries were engorged. After histopathological examination of these cases were diagnosed as necrotic haemorrhagic enteritis and colitis (Figure 1).

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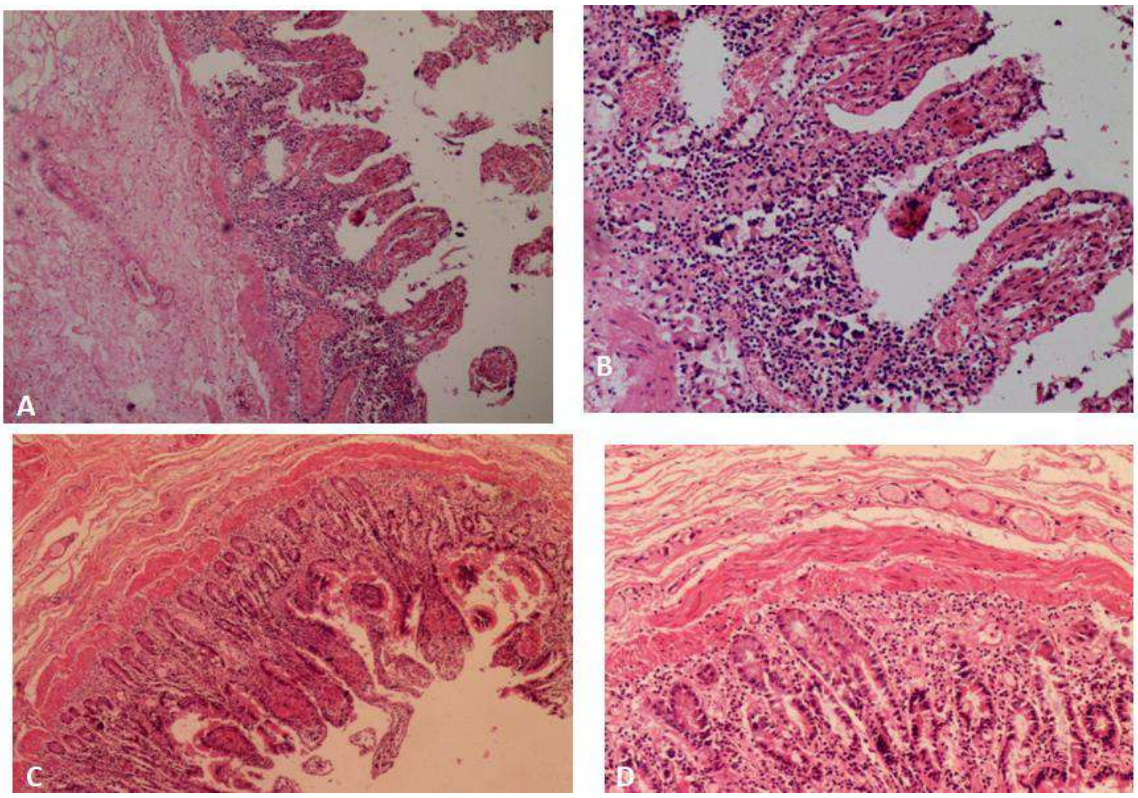

Figure 1: Photomicrograph showing denudation of the enterocytes, shortening of the villi, capillary congestion and infiltration mononuclear cells and macrophages in the lamina propria of mucosa as well as in the submucosal layer (A &C, 100 × ; B&D, 200 ×, H&E).

The haematological examination of blood samples (Table 1) indicated neutrophilia with (72.82 ± 4.06%) and Total Leukocyte Count (TLC) was 7.51 ±1.93 × 10^6 µ L^{-1}. Haemoglobin and the platelet content of all the horses were slightly lower and close to the lower margin, whereas total leukocyte was slightly higher in most of the horses. The haematological findings indicate normocytic hypochromic anemia in most of the horses.

		Serum Biochemistry								**Haematological Analysis**								
												Differential Leukocyte Count						
S. No.	**Horse Name**	**Urea (mg/dl)**	**Creatinine (mg/dl)**	**SGOT(IU/L)**	**SGPT (IU/L)**	**Total Protein (gm/dl)**	**Albumin (gm/dl)**	**Globulin (gm/dl)**	**Uric Acid (mg/dl)**	**Hb (gm/dl)**	**TLC (1000/ul)**	**N (%)**	**L(%)**	**E (%)**	**M (%)**	**B(%)**	**Platelet (1000/ul)**	**PCV (%)**
1	Gulab	79	1.9	219	14	5.7	2.7	3	0.5	10.3	5400	75	24	1	0	0	2.5	35
2	Rathore	13	1.6	244	12	5.8	2.4	3.4	0.3	11.2	6600	76	28	1	1	0	3.1	34

		1																
3	Paramveer	24	1.4	217	14	5.7	2.4	3.3	0.7	12.5	8300	66	33	1	0	0	2.4	44
4	Helen	24.8	1.2	276	14	5.3	2.7	2.6	0.3	11.8	7800	72	27	1	0	0	2.9	34
5	Bharat	28.8	1.4	300	15	5.9	2.5	3.4	0.3	11.5	7000	77	22	1	0	0	3.1	38
6	Raja	31.6	1.4	240	17	5.6	3	2.6	0.5	12	7100	71	27	1	1	0	5.2	41
7	Montena	34.4	1.5	256	16	6.1	3.4	2.7	0.7	15.4	5900	66	33	1	0	0	3.2	52
8	Fantasy	38.4	1.4	336	14	5	2.9	2.1	0.6	10.9	5500	73	26	1	0	0	1.8	37
9	Rustam	29.2	1.3	263	12	5	2.9	2.1	0.7	13.7	7200	72	26	2	0	0	2.4	46
10	Anant	32.8	1.3	214	14	4.8	2.8	2	0.1	11.7	5900	71	28	1	0	0	1.7	37
11	Booster	45.6	1.4	309	16	5.5	2.4	3.1	0.7	11.4	5800	78	21	1	0	0	2.5	35
12	Diamond	34	1.3	267	14	6.6	3.1	3.5	0.7	10.8	5600	70	29	1	0	0	2.4	36
13	Nagina	39.6	1.4	217	16	6.4	3	3.4	0.3	14.5	7900	72	27	1	0	0	1.9	48
14	Ramu	22.4	1.2	215	14	5.9	2.6	3.3	0.3	10	9500	72	27	1	0	0	2.4	35
15	Swastik	24	1.4	202	14	5.5	2.6	2.9	0.4	12.5	7900	68	30	2	0	0	2.3	43
16	Kamal	30.8	1.2	293	18	5.2	2.5	2.7	0.4	10	4800	77	22	1	0	0	3	35
17	Neelkanth	50.8	1.5	281	16	5.9	2.8	3.1	0.4	11.4	6500	76	22	1	1	0	3.8	41
18	Kaushal	29.2	1.1	327	19	6	2.7	3.3	0.4	10.4	7400	67	32	1	0	0	4.6	38
19	Badal	40	1.6	341	14	5.8	2.9	2.9	0.5	13.5	8300	70	29	1	0	0	4.5	47
20	Farishta	43.6	1.4	278	18	6.2	3	3.2	0.6	12.8	11700	78	21	1	0	0	1.7	40
21	Praval	66	1.3	329	18	6.7	3.2	3.5	0.4	13.1	6100	73	27	0	0	0	9	43
2	Rimjhim	35	1.5	274	19	5.8	2.7	3.1	0.4	14	6600	70	29	1	0	0	2.5	47

2		.6																
23	Rakhi	32.4	1.2	251	14	5.7	2.6	3.1	0.5	11.7	9600	80	19	1	0	0	3.6	38
24	Sol	42.8	1.5	281	6	6.3	2.4	3.9	0.8	11.7	8200	70	29	1	0	0	4	39
25	Shartaj	37.2	1.1	219	19	6.4	3.3	3.1	0.6	14.8	8000	74	24	2	0	0	4.4	49
26	Pawan	38	1.3	311	25	6.8	3.2	3.6	0.9	15.1	8700	71	26	1	2	0	1.8	52
27	Dara	68.8	1.7	291	16	5.9	2.8	3.1	0.7	12.8	13400	81	18	1	0	0	2.5	40

On bacteriological analysis of rectal swabs, a total of 137 bacterial isolates belonging to 17 *species* including *Acinetobacter calcoaceticus* (6), *Aeromonas schubertii* (1), *Bacillus cereus* (4), *Budvicia aquatica* (3), *Citrobacter freundii* (1), *Edwardsiella tarda* (37), *Lelliottia amnigenus* (22), *Escherichia coli* (27), *Hafnia alvei* (3), *Pantoea agglomerans* (4), *Pectobacterium cacticida* (1), *Pectobacterium rhapontici* (1), *Pseudomonas aeruginosa* (4), *Raoultella terrigena* (3), *Salmonella enteric* ssp. *arizonae* (2), *Yersinia enterocolitica* (14), and *Yersinia kristensenii* (4) were isolated (Table 2).

Table 2: Bacteria isolated from deep rectal swabs of horses and their resistance to herbal antimicrobials including ajowan oil (AO), holy basil oil (HBO), cinnamaldehyde (CNH), carvacrol (CC), lemongrass oil (LGO), thyme oil (TO), citral (Ctr), cinnamon oil (CO), and sandalwood oil (SWO).

***Species*, number of samples positive**	**Number of isolates**	**Number of isolates resistant to**								
		AO	**HBO**	**CNH**	**CC**	**LGO**	**TO**	**Ctr**	**CO**	**SWO**
*Acinetobacter calcoaceticus*5	6	1	0	0	2	1	2	0	3	1
Aeromonas schubertii 1	1	0	0	0	0	0	0	0	0	0
Bacillus cereus 3	4	0	0	0	0	0	0	0	1	0
Budvicia aquatica 2	3	2	0	0	2	0	1	0	3	2
*Citrobacter freundii*1	1	1	0	0	1	0	1	0	1	1
*Edawrdsiellatarda*13	37	16	0	0	35	1	32	3	35	16
Lelliottiaamnigenus 12	22	12	0	0	16	1	19	0	21	12
Escherichia coli 13	27	10	0	0	25	1	25	1	25	10
Hafnia alvei 3	3	1	0	0	3	0	3	0	3	1
*Pantoeaagglomerans*1	4	2	0	0	2	0	1	1	3	2
*Pectobacteriumcacticida*1	1	1	0	0	1	0	1	0	1	1
*Pectobacteriumrhapontici*1	1	1	0	0	1	0	1	0	1	1
Pseudomonas aeruginosa 1	4	4	1	3	4	4	4	4	4	4
*Raoultellaterrigena*2	3	2	0	0	3	0	3	1	3	2
Salmonella enterica ssp. *arizonae*2	2	2	0	0	2	0	1	1	2	2
Yersinia enterocolitica 6	14	5	0	0	13	0	10	0	14	5

*Yersinia kristensenii*3	4	4	0	0	3	0	1	0	4	4

Many of the bacteria were not isolated from more than a horse, but *E. tarda* and *E. coli* were isolated from 13 samples each, *Lelliottia amnigenus* from 12 samples, *Y. enterocolitica* from six samples, *A. calcoaceticus* from five samples, *B. cereus*, *H. alvei*, and *Y. kristensenii* from three samples each while *B. aquatica*, *R. terrigena*, and *S. enteric* ssp. *arizonae* were detected in two samples each.

Except amikacin and imipenem none of the antimicrobials tested including nine each of herbal antimicrobials (Table 2) and antibiotics (Table 3) inhibited all 137 isolates of bacteria from horse faecal samples. However, cinnamaldehyde, carvacrol, thyme oil, and ajowan oil inhibited the growth of 136, 134, 129, and 127 strains, respectively.

Table 3: Bacteria isolated from deep rectal swabs of horses and their resistance to antimicrobials including tetracycline (T), minocycline (M), amikacin (AK), nitrofurantoin (NF), ciprofloxacin (Cf), chloramphenicol (C), imipenem (I), ceftriaxone (CTR), ceftriaxone +tazobactam (CTT) and producing extended spectrum –lactamase(s)(ESBL).

***Species*, number of samples positive**	**Number of isolates**	**Number of isolates resistant to**									**ESBL producers**
		T	**M**	**AK**	**NF**	**Cf**	**C**	**I**	**CTR**	**CTT**	
*Acinetobacter calcoaceticus*5	6	2	1	0	4	2	2	0	4	2	4
Aeromonas schubertii 1	1	0	0	0	0	0	0	0	0	0	1
Bacillus cereus 3	4	0	0	0	0	0	3	0	1	0	3
Budvicia aquatica 2	3	1	1	0	1	0	0	0	1	0	2
*Citrobacter freundii*1	1	1	1	0	1	0	1	0	1	0	1
*Edawrdsiellatarda*13	37	11	20	0	19	0	11	0	16	2	27
Lelliottiaamnigenus 12	22	12	20	0	16	1	3	0	17	1	17
Escherichia coli 13	27	11	16	0	16	2	11	0	14	1	25
Hafnia alvei 3	3	0	2	0	3	0	1	0	3	0	3
*Pantoeaagglomerans*1	4	1	0	0	1	0	0	0	1	0	3
*Pectobacteriumcacticida*1	1	0	1	0	1	0	1	0	1	0	1
*Pectobacteriumrhapontici*1	1	1	1	0	1	0	1	0	1	0	1
Pseudomonas aeruginosa 1	4	4	4	0	2	0	0	0	2	0	2
*Raoultellaterrigena*2	3	2	3	0	3	0	0	0	3	0	3
Salmonella enterica ssp. *arizonae*2	2	2	2	0	2	0	2	0	2	0	2
Yersinia enterocolitica 6	14	3	5	0	6	2	4	0	3	2	9
*Yersinia kristensenii*3	4	1	4	0	4	0	0	0	1	0	4

Among herbal antimicrobials (Table 2), in order of their antimicrobial efficacy on bacterial isolates from horse rectal swabs the most effective was cinnamaldehyde (99.27%) followed by carvacrol (97.81%), thyme oil (94.16%), ajowan oil (92.70%), cinnamon oil (91.97%), holy basil oil (53.28%), citral (23.36%), lemongrass oil (17.52%) and sandalwood oil (9.49%).

Among antibiotics the most effective were amikacin and imipenem inhibiting all 137 bacterial isolates followed by ciprofloxacin (94.89%), ceftriaxone + tazobactam (94.16%), chloramphenicol (70.80%), tetracycline (62.04%), ceftriaxone (48.18%), nitrofurantoin (41.61%) and minocycline (40.88%). A total of 108 (78.83%) of the 137 bacterial isolates were identified as ESBL producers (Table 3).

There was no significant difference in the susceptibility of isolates of different bacteria detected in faecal sample of horses for most of the herbal antimicrobials and antibiotics.

However, lemongrass oil resistance was more common in *E. tarda* isolates than in *L. amnigenus* (p, 0.02) isolates. However, tetracycline resistance was more common in *L. amnigenus* isolates than in *Y. enterocolitica* (p, 0.05) isolates. Minocycline resistance was also more common in *L. amnigenus* isolates than in *E. tarda* (p, <0.01), *E. coli* (p, 0.01) and *Y. enterocolitica* (p, <0.01) isolates. *Yersinia enterocolitica* isolates were more often resistant (p, 0.02) to minocycline than *E. tarda* isolates. Chloramphenicol resistance was more rampant in *E. coli* strains (p, 0.04) than in *L. amnigenus* strains. Ceftriaxone resistance was more commonly seen among isolates of *L. amnigenus* than among isolates of *E. tarda* (p, 0.01) and *Y. enterocolitica* (p, <0.01). The ESBL production was more commonly seen among *E. coli* isolates than in isolates of *E. tarda* (p, 0.05) and *Y. enterocolitica* (p, 0.02).

A total of 137 bacterial isolates belonging to 17 *species* of enteric bacteria from equine faecal samples were classified in to 89 ASTs based on their susceptibility to different herbal antimicrobials and antibiotics. Only one isolate of *P. agglomerans* was susceptible to all nine antibiotics and herbal antimicrobials. Of 89 ASTs, 62 had only one isolate each belonging to them.

The AST-39 (resistant to HBO, LGO, Citral, SWO, tetracycline, minocycline, nitrofurantoin, cefotaxime, and producing ESBL) was the most common type and 10 isolates (2 *E. tarda*, 5 *L. aminigenus*, 1 *E. coli* and 2 *R. terrigena*) belonged to this AST. Two ASTs (59 and 60) had 4 isolates each. The AST-59 had one isolate each of *E. tarda*, *L. amnigenus*, *E. coli* and *H. alvei* while AST-60 had two isolate each of *L. amnigenus* and *E. coli*. Three isolates belonged to each of the 9 ASTs (12, 26, 30, 38, 62, 65, 66, 83 and 85) while 15 ASTs (25, 27, 28, 29, 34, 41, 43, 48, 51, 54, 56, 58, 64, 70, 88) had two isolates each.

Diversity among isolates belonging to the same *species* from horses reared in the same stable was evident by the fact that no more than five isolates (*L. amnigenus*) of any *species* belonged to the same AST. The highest numbers of ASTs were 34 in a single *species E. tarda* isolates followed by 27 *E. coli* isolates belonging to 25 ASTs, 14 *Y. enterocolitica* isolates belonged to 14 ASTs and 22 *L. amnigenus* isolates belonged to 16 ASTs.

Based on the detection of enteric bacteria from multiple samples, the serum from each of the horse was tested for determining agglutination titre for different bacteria isolated from the horse. Besides, each horse serum was also tested for agglutination titres for *Y. kristensenii*, *Y. enterocolitica*, *E. tarda* and *L. amnigenus* heat-killed antigens, irrespective of the isolation from the horse.

From six rectal swabs of horses (Horse no. 1, 2, 14, 16, 20, 26) none of the four most commonly occurring bacteria was detected. But in serum samples of those horses too agglutination titres were quite high (≥ 1280) against *Y. enterocolitica*, *E. tarda* and *L. amnigenus*. At the same time, agglutination of *E. coli*, *R. terrigena*, *H. alvei* and *P. agglomerans* was either not detected or titres were very low (<10) even in those horses from those these bacteria were detected except in serum from one horse each having *E. coli* and *H. alvei* agglutination titres 320 and 80, respectively.

The highest average titre in horse sera samples (Table 4) was against *Y. enterocolitica* (4646 ± 2514), followed by *E. tarda* (3058 ± 2605), *Y. kristensenii* (2299 ± 1804) and *L. amnigenus* (2216 ± 2399). However, the highest titres against *Y. enterocolitica* were recorded 10240 in the serum of three horses and 5120 in 15 serum samples, against *Y. kristensenii* the highest tire was 5120 in seven serum samples. The highest titre against *E. tarda* was 10240 in two serum samples and 5120 in six serum samples, and the highest agglutination titre for *L. amnigenus* was estimated 10240 in two sera but none had titre equivalent to 5120. There were only three sera (from horse no. 8, 16, 17) samples having titre 1280 against *Y. enterocolitica*, *E. tarda*, *Y. kristensenii* and *L. amnigenus*.

From two soil samples collected from the stable a total of 13 bacteria belonging to 12 different ASTs (29, 54, 58, 90, 91, 92, 94-99) were identified. The isolates belonged to *Aeromonas bestiarum* (1), *Bacillus coagulans* (1), *B. mycoides* (1), *L. amnigenus* (7), *L. sphaericus* (2) and *Pseudomonas aeruginosa* (1). One *L. amnigenus* isolated from a soil sample had a similar AST pattern (58) as the one isolated from a horse and from a filtered water sample

(RW), however, three other *L. amnigenus* isolated from soil samples belonged to ASTs detected in other bacterial isolates but not in *L. amnigenus* isolates from horse rectal swabs, and one *L. amnigenus* isolate had altogether different AST (90).

Table 4: Agglutination titres of horse serum samples against *Edwardsiellatarda, LelliottiaamnigenusYesinia enterocolitica,* and *Y.kritensenii*

S. No. Of Horse	Agglutination titres against formalin inactivated bacteria			
	***Edwardsiella tarda* (MH5P)**	***Lelliottia amnigenus* (MH24NH2)**	***Yersinia enterocolitica* (MH1P3)**	***Y. kritensenii* (MH1P1)**
1	1280	640	5120	640
2	640	1280	5120	1280
3	640	320	10240	640
4	1280	1280	5120	1280
5	2560	1280	5120	640
6	2560	1280	5120	1280
7	1280	1280	5120	2560
8	1280	1280	5120	640
9	1280	1280	5120	1280
10	1280	1280	5120	1280
11	5120	1280	10240	640
12	2560	1280	10240	2560
13	2560	1280	1280	2560
14	2560	1280	2560	640
15	5120	1280	5120	1280
16	1280	1280	1280	640
17	1280	1280	1280	1280
18	5120	1280	2560	5120
19	2560	2560	5120	5120
20	1280	2560	1280	1280
21	5120	2560	2560	5120
22	5120	2560	2560	2560
23	640	2560	2560	5120
24	2560	2560	5120	5120
25	5120	2560	5120	5120
26	10240	10240	5120	5120
27	10240	10240	5120	1280
Average	**3058**	**2216**	**4646**	**2299**
STDV	**2605**	**2399**	**2514**	**1804**

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Two water samples, one coming from a deep bore well and another of filtered potted (stored) water, yielded six and five isolates of bacteria belonging to nine ASTs (48, 58, 61, 90,93, 100-103). The isolates from water samples were identified as *Alcaligenes faecalis* (1 SW), *B. coagulans* (1 FW), *L. amnigenus* (3 SW, 1 RW), *P. aeruginosa* (1 SW and 2 RW), *Raoultella terrigena* (2 RW). Except for an isolate each of *L. amnigenus* from soil and RW no two isolates had the AST patterns (58) similar to those detected in horse rectal swabs. Besides, two *P. aeruginosa* isolates, one each from SW and RW, belonged to the same AST-102.

On comparison of antibiotic susceptibility of bacteria isolated from horses and soil to different antimicrobials no significant difference was evident except for a higher proportion of resistant isolates to cinnamon oil (p, <0.01) and susceptible to cefotaxime (p, 0.01) in isolates from soil samples than those from equine rectal swabs.

On comparison of antibiotic susceptibility of bacteria isolated from horses and water to different antimicrobials no significant difference was evident except for a higher proportion of resistant isolates to ajowan oil (p, <0.01), carvacrol (p, <0.01), thyme oil (p, <0.01), and cinnamon oil (p, <0.01) from water samples than those from equine rectal swabs.

On comparison of antibiotic susceptibility of bacteria isolated from water and soil samples to different antimicrobials no significant difference was evident except for a higher proportion of resistant isolates from water samples to ajowan oil (p, 0.03), carvacrol (p, <0.01), thyme oil (p, <0.01), and tetracycline (p, 0.04) than those isolated from soil samples.

Isolates of *L. amnigenus* isolated from water and soil were significantly more often resistant to cinnamon oil (p, 0.01) but more often susceptible to cefotaxine (p, 0.01) than those isolated from equine rectal swabs.

After investigation in feeding of ajowan (*Tachyspermum ammi*) seeds to horses for a week (100 gm in 100 gm jaggery per day per horse) were fed. Change of stable sand after decontamination of cleaned stable with lime was recommended. All horses recovered from mild illness and signs of colic they were showing intermittently, and after January 2023 till date no case of colic or other sickness is reported from the horses.

Discussion

Marked neutrophilia with marginally higher TLC in all the horses indicated the presence of some inflammatory cause, most probably the invasion of bacteria [10]. The observation also corroborated with necrotic and haemorrhagic enterocolitis in post-mortem findings and also with detection of high levels of antibodies to *Y. enterocolitica*, *E. tarda*, *Y. kristensenii*, and *L. amnigenus* in all the horses. The results of bacterial isolation and antibody titres in horse serum samples for *Y. enterocolitica*, *E. tarda*, *Y. kristensenii*, and *L. amnigenus* indicated that these bacteria might be acting in concert to induce enterocolitis in horses.

Observation on the susceptibility of bacteria to herbal antimicrobials specially cinnamaldehyde (99.27%), carvacrol (97.81%), thyme oil (94.16%), ajowan oil (92.70%), and cinnamon oil (91.97%), is in corroboration of earlier observations on bacteria causing clinical infections in animals [11,12]. Though amikacin and imipenem were the effective antibiotics on all putative pathogens identified, the selection of ajowan seeds as treatment option was based on our experience with this herb curing most of the bacterial infections in the gut of animals. The ajowan seeds are easily and economically available in most of the general grocery stores in Indian markets. Moreover the effective antibiotics amikacin and imipenem are costly and usually not preferred to be used in horses. The success of treatment again proved the therapeutic efficacy of the herb ajowan seeds.

Yersinia enterocolitica, a gram-negative bacterium of the Enterobacteriaceae family, primarily a gastrointestinal pathogen is reported to causing septicaemia disorders in several *species* including horses [13]. It is considered to be a widespread bacterium in nature colonizing pigs, sheep, cattle, horses, rodents, and human intestines [14]. Though *Y. enterocolitica* is known to cause mild to severe gastroenteritis in humans disappearing within 1-3 weeks,

rarely reported to cause gastroenteritis or enterocolitis in horses [15,16], thus it seems to be the first report of *Y. enterocolitica* associated with enterocolitis in horses. The association of *Y. enterocolitica* with lethal form of enterocolitis might be the result of mixed infection with other bacteria. *Yersinia frederiksenii* formerly known as atypical *Y. enterocolitica* or *Y. enterocolitica*-like, isolates are commonly considered as non-pathogenic commensals and can be detected in stool cultures of healthy subjects; however, several cases of the diarrhoeic disorder have been reported in humans [17].Very high agglutination titres to *Y. frederiksenii* in all 27 horses indicated that it might have invaded the horse system and might be contributing to pathogenesis of enterocolitis. The *E. tarda* was isolated from several horses and high agglutinations titres in the serum of horses indicated its association with enterocolitis pathogenesis. Though *E. tarda* is primarily a fish pathogen causing septicemic diseases associated with loss of skin pigmentation, abdominal bloat, and haemorrhagic skin lesions and rectal prolapsed with ascites, peritonitis, hepatic, splenic and renal congestion in fishes [18], also reported to affects human and animals [19,20,21]. However, *E. tarda* has rarely been isolated from clinical cases in equids except recently from a case of wound infection [21]. The other important bacterium which was associated with enterocolitis in horses was *L. amnigenus*. It is one of the members of Enterobacteriaceae, often colonizing in intestines of animals. *Lelliottia amnigenus* (formerly known as *Enterobacter amnigenus*) is reported common in equine faecal samples [22,23] but has rarely been reported to cause a clinical infection in horses as seen in the present investigation.

The other bacteria isolated and identified from horse rectal swabs but not incriminated to cause enterocolitis in this study are also common including *Salmonella* causing salmonellosis. Salmonellosis, a common disease associated with enteritis, enterocolitis, septicaemia and abortions in horses is often caused by *S. enterica* ssp. enterica serovars [2]. However, *S. enterica* ssp. arizonae has rarely been reported to cause ulcerative keratitis [24], and abortion [25] in equids. The other important bacteria were *R. terrigena*. Strains of *Raoultella* (*R. ornitholytica*, *R. planticola*, *R. terrigena*) and *Klebsiella* not only share ecological, biochemical, clinical, and microbiological features but often indistinguishable in clinical settings, that is why both were grouped under the same genus earlier, *Klebsiella*. In the last two decades, raoultellosis has gained importance as an emerging disease. *Raoultella* strains are ubiquitous, being found in plants, water, and soil, and are known to colonize gastrointestinal tract of humans and animals [26] including horses [27]. *Acinetobacter calcoaceticus* isolated from horses in the study might be just an opportunistic colonizer in gastrointestinal tract of horses. *Acinetobacters* are often the neglected pathogens not only in horses but in the whole veterinary practice [28]. However, several nosocomial infections of horses are on record especially of pneumonia and catheter-related infection [29]. Though *A. calcoaceticus* may occasionally causes of septicaemia [30], and abortions [31] in horses, its asymptomatic prevalence in horses was indicated in Poland on a horse farm when almost one-third of workers at the farm had antibodies to *A. calcoaceticus* [32]. Though in the present study *A. schubertii* was isolated as a non-pathogenic isolate, aeromonads are commonly reported to causing abortion, wound infection, and diarrhoea in animals [33] and *A. schubertii* is a common cause of human wound infection [34]. However, isolation of *A. schubertii* may be important from zoonotic aspect but it has rarely been isolated from healthy or sick horses [31]. *Bacillus cereus* often causing food poisoning characterized by diarrhoea and emesis in human beings is reported to cause dermatitis [35] and wound infections [36] in horses. It has rarely been isolated from the faeces of apparently healthy or sick horses. Due to food poisoning potential, the presence of *B. cereus* in horse faeces is important from the public health point of view. *Budvicia aquatic*, isolated from two horses in the study are often considered non-pathogenic bacteria and are commonly detected in water, faeces and sewage [37]. However, in immune-compromised and stressed humans it is reported to cause sepsis [38]. The *C. freundii*, though common in the environment was isolated from a single horse rectal swab. It occasionally induces illness in human and animals. It is one of the major opportunistic pathogen and represents up to 29% of all

opportunistic hospital infections [39]. In horses it is reported to cause endocarditis [40], neonatal septicaemia [41], endometritis [42,43], ulcerative keratitis [44], arytenoid chondropathy [23], arthritis [44], and also isolated from various other equine clinical samples [45,46]. The most commonly isolated bacteria were *E. coli* and it has been reported as the most common bacteria causing gastrointestinal problems in horses [2]. However no antibody detection to *E. coli* in most of the horses indicated it's non-pathogenic commensally presence. Another rarely isolated bacteria from horses identified in this study was *H. alvei*. It is another member of the Enterobacteriaceae family, and is often reported in association with emerging antimicrobial-drug-resistance [27]. It may cause abortions in mares [47]. *Pantoea agglomerans* isolated from a horse sample is an important pathogen reported to cause endometritis [42], and abortion in mares [46,48]. *Pectobacterium* strains earlier classified in genus *Erwinia* are mostly considered as plant pathogens and commensals in soil have rarely been reported to cause wound infections in horses [49] and their isolation from rectal swabs of two horses is of not much significance. However, the detection of *P. aeruginosa* from faeces is not uncommon in animals; it was detected from the sample of a horse. It is known to cause a variety of systemic and soft tissue infection in equids [50]. Even they cause abortions and chronic endometritis in mares [31,42,51]. It is considered as venereally transmitted infection in horses [52] thus important bacteria to be taken seriously.

Conclusion

The study concluded that bacteria often considered rare and present commensally may also cause severe enterocolitis in horses. The bacterial enterocolitis characterized by neutrophilia was associated with mixed infection by four bacteria (*E. tarda, L. amnigenus, Y. enterocolitica*,*Y. kristensenii*) rarely reported in horses. The success of ajowan (*T. ammi*) seeds in treating enterocolitis in horses may be a promising observation and the herb can be used in veterinary therapeutics being an economic alternative to costly antibiotics.

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