Outbreak of Abortions and Infertility in Thoroughbred Mares Associated with Waterborne *Aeromonas hydrophila*

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Abstract At a thoroughbred equine breeding farm near Hissar (Haryana), three mares aborted in their seventh month of pregnancy. The vaginal swabs of all aborted mares, and stomach contents, heart blood, liver, spleen and placenta of aborted fetuses yielded pure culture of *Aeromonas hydrophila*. In addition, *A. hydrophila* was also isolated from the vaginal swabs of three repeat breeding mares and faecal sample of a diarrheic foal. The source of infection was possibly water supply as all the water samples collected from taps, mother tank and storage tank were found to be positive for *A. hydrophila*. The antibiogram of all the isolates was similar showing resistance to ampicillin, carbenicillin, gentamicin, kanamycin and amikacin but sensitive to chloramphenicol, ciprofloxacin, cefuroxime, ceftriaxone, cotrimoxazole, cotrimazine, nitrofurantoin, streptomycin and tetracycline. All the 20 sera samples collected from three aborted and three repeat breeding, and eight in-contact mares, a diarrheic foal, three cows and two male buffaloes maintained at the same farm contained antibodies against *A. hydrophila* with titres ranging from 80 to 640. The water supply was instantly chlorinated using 0.05% sodium hypochlorite for three consecutive days and all the culturally positive mares were treated with intravaginal administration of 1 g ciprofloxacin, while the foal was given nitrofurantoin

for three days. After one month, *A. hydrophila* could not be isolated either from mares or from their environment and antibody titre in all the seropositive animals showed a declining trend. Later, all the aborted and repeat breeding mares were confirmed to be pregnant. Thus, the present study indicated that water-borne *A. hydrophila* might be associated with equine abortions and infertility, and diarrhea in newborn foals.

Keywords *Aeromonas hydrophila* · Mares · Abortions · Foal diarrhea · Repeat breeding

Introduction

The most common etiological agents of abortion in equids include equine viral arteritis (EVA), equine herpes virus-1 (EHV-1), *Streptococcus zooepidemicus* and *Salmonella enterica* subsp. *enterica* serovar Abortusequi [1, 2]. Besides, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Taylorella equigenitalis* (CEM), *Streptococcus equisimilis*, *Leptospira pomona*, *Corynebacterium equi*, *Brucella abortus*, *Actinobaccillus* spp. and *Rhodococcus equi* may also cause abortion in the pregnant mare either alone or in concert with other pathogens [2, 3]. Aeromonads are often considered to be non-pathogenic for animals but animals may act as carriers and faecal shedders [4, 5]. Gray [4] isolated *Aeromonas hydrophila* from feces of normal horses (7 of 110, 6.4%), pigs (11 of 115, 9.6%), sheep (10 of 111, 9.0%), and cows (26 of 123, 21.1%). Although, rarely, *A. hydrophila* has also been reported to cause abortion in mares from abroad [6, 7], but it appears to be first report from India.

Food and water are the major sources of infection for terrestrial animals because aeromonads are common in

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urban effluents, natural water, and potable drinking water and even in chlorinated drinking water supplies [8–10]. *Aeromonads* may grow in water distribution systems as biofilms after an initial contamination [11], where they may be protected from disinfectants [12, 13]. The findings of the present investigation reveal association of water borne *A. hydrophila* with abortion, infertility and diarrhea in equids.

Materials and Methods

The samples consisting of the allanto-chorion, stomach contents, lung, spleen, heart blood and liver from the aborted fetuses were collected and sent by the clinician in sterile polythene bag on ice. Besides, cervico-vaginal swabs and blood samples from all the aborted and repeat breeding mares were also collected aseptically and transported on ice [14] to the laboratory. Samples were analyzed for EHV-1 and EHV-4 DNA using polymerase chain reaction (PCR) to amplify [15]. For the isolation of bacteria, all the samples were cultured on 5% blood agar (BA) and eosin methylene blue (EMB) agar (Hi-Media, Mumbai) plates in duplicate. One set of the plates was incubated in 5% CO₂ and other under aerobic conditions at 37°C for 24 h. The plates were examined for pathogenic bacteria and isolates were identified on the basis of their morphological, cultural and biochemical characteristics [16]. Sera from all the blood samples were separated [14] and stored at 4°C, while clots were processed for the isolation of bacteria as described above [16].

Having identified all the isolates recovered from the above clinical samples as *A. hydrophila*, venous blood, and rectal and vaginal swabs from all the 14 mares including three aborted, three repeat breeders and eight in contact mares were collected. In addition, venous blood and faecal samples from one diarrheic foal (15-day-old), three cows, and two male buffaloes maintained at the same farm were also collected aseptically and subjected to bacteriological examination as above [14]. In addition, specific alkaline peptone water enrichment and streaking on ampicillin dextrin agar (ADA Hi-Media, Mumbai) plates for the isolation of *Aeromonas* [14, 16] was attempted from all samples.

Environmental samples from the stud farm including soil, water (from taps in rooms of aborted mares, storage tanks and mother tank) and feed samples were examined for the presence of aeromonads [17] and isolates were characterized as described above [14, 16].

As a follow up study, samples from all the above sources were again collected after approximately one and six months post first collection and examined as described above [14–17].

Water samples were subjected to *Aeromonas* count using membrane filter method, typical colonies were counted and randomly checked for confirmation [14, 16].

For serological examination, all the sera samples were tested for salmonellosis and brucellosis using commercial antigens (IVRI, Izatnagar, Bareilly) of *S. enterica* serovar Abortusequi and *Brucella abortus* through the micro-agglutination test (MAT) method [18]. *Aeromonas hydrophila* agglutinating antibodies in sera samples were detected using antigen made from three *A. hydrophila* isolates (one each from fetus, mares and water) using MAT [18].

The water supply was chlorinated for three consecutive days using sodium hypochlorite @ 0.05% of water volume. All the six culture positive mares were treated with intravaginal ciprofloxacin (1 g/day); the foal was given nitrofurantoin (300 mg/day in two divided doses) for three days.

Antimicrobial Sensitivity Assay

Antimicrobial sensitivity of *Aeromonas* isolates was determined by the disc diffusion method on Muller Hinton agar No. 4 using amikacin 10 µg (AK), ampicillin 10 µg (Am), carbenicillin 10 µg (Cb), chloramphenicol 30 µg (C), ceftriaxone 25 µg (Ci), cefuroxime 10 µg (Cu), ciprofloxacin 5 µg (Cf), cotrimoxazole 25 µg (Co), cotrimazine 25 µg (Cm) nitrofurantoin 300 µg (Nf), gentamicin 10 µg (G), kanamycin 30 µg (K), streptomycin 10 µg (S), and tetracycline 30 µg (T) discs (Hi-media, Mumbai). Based on zone of growth inhibition, isolates were classified as sensitive or resistant according to CLSI standards [19]. A reference *E. coli* K12 strain (E-382), sensitive to all antimicrobial agents, was used as control.

Results

None of the mares were found to be positive for EHV-1 or EHV-4 infection. However, oxidase and catalase positive isolated colonies, having similar morphology (circular and β-haemolytic on BA plates and reddish, raised, circular colonies on EMB plates) were observed on plates inoculated directly from the tissue impressions and also on those inoculated from peptone water cultures of spleen, liver, stomach contents and heart blood homogenates of the aborted fetuses. Similar colonies were seen on plates inoculated with placental homogenates and vaginal swabs received in the laboratory for cultural examination. On the basis of their morphological, cultural and biochemical characteristics of all the isolates were identified as *A. hydrophila*.

Aeromonas hydrophila was again isolated from all of the samples collected later from the vagina of three aborted

and three repeat breeding mares, and a faecal sample from a foal with diarrhoea. The water samples from taps, storage tanks and the mother tank also yielded *A. hydrophila*, but none of the samples collected from tube-well water, feed, soil, or faecal samples of mares, cows or buffaloes were found to be positive for the presence of *A. hydrophila*.

A total of 27 samples including cervicovaginal swabs (6), water samples (5), faeces of a diarrhoeic foal (1), placentae from aborted mares (3) and foetal contents (stomach contents, heart blood, liver and spleen) were positive for *A. hydrophila*. *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp. and *Streptococcus* spp. (α -haemolytic and non-haemolytic) isolates were recovered from majority of the faecal samples, cervico-vaginal swabs, soil samples and placentae, but not from water samples or aborted foetal contents. Besides *A. hydrophila*, water samples also yielded *Pseudomonas* spp. From feed samples, only *Enterobacter* spp, *Staphylococcus* spp. *Streptococcus* pp. and *Pseudomonas* spp. could be isolated.

All the isolates of *A. hydrophila* were resistant to ampicillin, carbenicillin, gentamicin, kanamycin and amikacin, but sensitive to chloramphenicol, ciprofloxacin, cefuroxime, ceftriaxone, cotrimoxazole, cotrimazine, nitrofurantoin, streptomycin and tetracycline, thus, showing multi-drug-resistance.

Water samples from water taps in stables had the highest *A. hydrophila* count ($6.7 \pm 1.2 \times 10^4$ CFU ml⁻¹), while it

was the least in mother tank samples ($2.3 \pm 0.8 \times 10^3$ CFU ml⁻¹). In water sample collected from two different storage tanks, the count was $2.4 \pm 0.7 \times 10^3$ and $3.3 \pm 0.9 \times 10^3$ CFU ml⁻¹.

None of the serum sample was positive for *Salmonella* or *Brucella* antibodies however, sera samples of all the aborted and repeat breeding mares yielded high titres of *A. hydrophila* agglutinins. Although no aeromonad could be isolated from any of the apparently healthy mares, cows or buffaloes, they were found to be seropositive (Table 1). After treatment of aborted and repeat breeding mares with antibiotics and chlorination of water supplies, the agglutinin titres showed a declining trend in all the animals. Moreover, *A. hydrophila* could not be isolated either from water or from any animals after treatment. Later, all the aborted and repeat breeders also conceived as confirmed by the farm veterinarian.

Discussion

Infertility and abortions caused by infectious agents [2, 3] are the major threats to most of the equine breeding stud farms. Although a number of different bacteria have been associated with abortion and infertility in breeding mares, association of *Aeromonas hydrophila* with these ailments has rarely been reported. Forga-Martel and coworkers

Table 1 *Aeromonas hydrophila* agglutinin titres in sera samples of animals exposed to *A. hydrophila* contaminated water

Type of animal	Animal number	Health status	Antibody (agglutinin) titres on			
			11-12-06	19-12-06	23-01-07	20-6-07
Mare	181	Aborted on 11th Dec 06	160	320	160	<20
Mare	195	Aborted on first Dec 06	320	640	160	<20
Mare	185	Repeat breeder	320	320	80	<20
Mare	186	Repeat breeder	320	320	160	<20
Mare	213	Repeat breeder	320	320	160	<20
Mare	197	Healthy, pregnant	Not tested	80	20	<20
Mare	201	Healthy, pregnant	Not tested	160	20	<20
Mare	202	Healthy, pregnant	Not tested	160	20	<20
Mare	203	Healthy, pregnant	Not tested	320	20	<20
Mare	204	Healthy, empty	Not tested	160	20	<20
Mare	205	Healthy, empty	Not tested	320	40	<20
Mare	206	Healthy, empty	Not tested	320	20	<20
Mare	207	Healthy, foaled	Not tested	160	80	<20
Cow	C1	Healthy, pregnant	Not tested	160	20	Not tested
Cow	C2	Healthy, pregnant	Not tested	80	80	Not tested
Cow	C3	Healthy, empty	Not tested	320	20	Not tested
Buffalo	B1	Healthy	Not tested	320	20	Not tested
Buffalo	B2	Healthy	Not tested	640	40	Not tested
Foal	F1	Diarrheic since fourth day of birth	Not tested	80	40	<20

[6] reported a case of infectious abortion caused by *A. hydrophila* in a mare and traced the source of infection to an adjacent dairy farm. However, in India, it appears to be first report on association of *A. hydrophila* or any other aeromonad with abortion or infertility in animals including mares.

In the present investigation, water supply did not appear to be recently contaminated with faeces as no faecal coliform was detected. However, *Aeromonas* count was much higher ($6.7 \pm 1.2 \times 10^4$ CFU ml⁻¹) than the maximum permissible limits of aeromonads in drinking water, i.e., 200 CFU/100 ml at 25°C [20]. It might be due to the fact that faecal coliforms could only be detected when contamination is recent while aeromonads can persist as biofilms for months together after initial contamination either of faecal or any other origin [11, 12]. It seems that heavily contaminated water might have served as the source of *A. hydrophila* infection to farm animals resulting in persistent infection on the equine farm, eventually leading to abortions and infertility in mares and diarrhea in foals. This observation is in concurrence with earlier studies revealing water as the most common source of *Aeromonas* spp. infection in human with gastroenteritis [10, 20].

The presence of anti *A. hydrophila* agglutinins in sera samples of all the animals on this farm including equids, cows and buffaloes, indicates that they all had an exposure to *A. hydrophila* and that they might have suffered with sub clinical or unapparent infection. Increasing agglutinin titres after abortion in mares may possibly be due to aggravation of infection because of physiological stress after parturition [21]. Despite ongoing disease, low titres in the foal might be due to its poor immune response to infection and/or low antibody titre in the mother's serum [21]. Declining anti-*A. hydrophila* titres, conception of repeat breeding and aborted mares after their treatment and chlorination of water supply line suggested that *A. hydrophila* could have been responsible for the problem on the farm. Association of *A. hydrophila* with infertility in mares or any other animals is rare and needs further experimentation to understand the pathogenesis.

Resistance to ampicillin and carbenicillin in all of the isolates under study can be ascribed to the observation that aeromonads, barring a few exceptions [17], are often resistant to β -lactam antibiotics [22]. All of the isolates were also resistant to aminoglycosides which were in common use for the treatment at the farm. Antimicrobial drug sensitivity of *A. hydrophila* isolates is in concurrence of earlier reports [17, 22].

Aeromonas hydrophila as cause of diarrhoea in foals is not surprising because it is known enterotoxigenic enteropathogens of warm blooded animals [23]. Although enterotoxin production was not tested in the study, all

being haemolytic might be enterotoxigenic too because of co-occurrence of both the characters [24].

Thus, on the basis of our findings, it can be deduced that *A. hydrophila*, though an opportunistic pathogen, might be associated with diarrhea in foals, abortion and infertility in mares even without apparent septicemia or any other manifestations. The presence of *A. hydrophila* agglutinins in sera samples of animals can be used as an indicator of infection in farm animals.

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