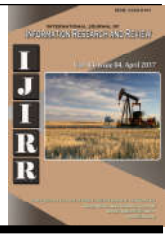
IJIRR

International Journal of Information Research and Review
Vol. 04, Issue, 04, pp.3972-3976, April, 2017



Research Article

AN INVESTIGATION OF *ENTEROBACTERAGGLOMERANS (PANTOEAAGGLOMERANS)* AND *KLEBSIELLAPNEUMONIAE* FROM ABORTED MARES

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ARTICLE INFO

Article History:

Received 20th January, 2017
Received in revised form
18th February, 2017
Accepted 19th March, 2017
Published online 30th April, 2017

Keywords:

Abortion; *Enterobacteragglomerans; Klebsiella pneumonia*;
Mare; Najran;
Saudi Arabia;Stillbirth.

ABSTRACT

*Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumonia*are opportunistic Enterobacteriaceae, lactose fermenting and a Gram-negative rod. It is apprisedas commensal pathogens of animal and human.Isolation and identification of *Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumoniae* from aborted mares were depended on the characters and features of colonies on blood agar plate and McConkey agar plates in addition to biochemical tests to isolated colonies from aborted cases according to Microbact™ Gram-negative system. Five mares have been aborted.Pure colonies of *Enterobacteragglomerans (Pantoeaagglomerans)* were isolated from two mares while pure colonies of *Klebsiella pneumonia* were isolated from one mare. Mixed infection of *Enterobacteragglomerans (Pantoeaagglomerans), Klebsiellapneumoniae, Escherichia coli and Citrobacter spp.* was isolated from two-aborted mare. Abortion rate in mares was 5.9 % (5/85). Isolated colonies of *Enterobacteragglomerans (Pantoeaagglomerans)* were susceptible to Ciprofloxacin (CIP) (100%) whilst isolated colonies of *Klebsiella pneumonia* were susceptible to Ceftriaxone (CRO) (100%). Both of two species were resistant to Ampicillin (AMP), Gentamicin (CN) and Tetracycline (TE).The isolation of *Enterobacteragglomerans(Pantoeaagglomerans)* and *Klebsiellapneumoniae* from vaginal cervico swabs after mare's abortion suggests them to be a cause of abortion in mares.

INTRODUCTION

Elevated economic industry loss of horse farm is caused by Abortion, stillbirth andequine deaths (Jeffcott, 1973).The placentitis is main cause of still birth and abortion in mares but there are another many causes of abortions (Rooney, 1976). Hormonal, genetic, viral, fungal, mycoplasmal, rickettsial and bacterial are common causes of Abortion in equines (Fontaine, 1993; Garg, 1966). Depending on locality, climate and breeding plan role of various etiological factors vary greatly, however infectious causes are alwaysresponsible for more than 50% abortions (Fontaine, 1993 and Varshney, 1994). Among the infectious causes, bacteria are invariably present either as primary cause or as secondary invader (Garg, 1986). In previous studies,placentitis in horses has been commonly caused by different pathogens specially Enterobactersp., *K. pneumonia, E. coli S. zooepidemicus,* fungi *and P.aeruginosa*(Swerczek, 1986; Whitwell, 1988).

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Abortion during 7-10th month of pregnancy in mares due to *E. agglomerans*has been occurred from placentitis and fetal death as result of occlusion of placental fetal blood supply (Hong, 1993). Plasmid profilesof*Klebsiella pneumonia* were isolated from the genital tract of stallions, the genital tract of mares with metritis and extra-genital sites of healthy mares (Kikuchi, 1995).The findings of the present investigation reveal association of *Entrobacteragglomerans* (*Pantoeaagglomerans*) and *Klebsiellapneumoniae* with the primary cause of abortion in late pregnancy in mares.In addition, it was to perform the isolation, identification and antimicrobial susceptibility pattern of *Pantoea* (*Enterobacter*) *agglomerans* and *Klebsiellapneumoniae* strains

MATERIAL AND METHODS

History information: During summer season, 2014 in Najran region, south area in Saudi Arabia, abortion in 5 mare (British equine breed) at 7 month of pregnancy and fetuses were found dead and theywere occurred in mare breeding farm (85 Mares) in El forresia club. Stallion was came from Riyadh.

Current investigation region: The current investigation geographic area wasNajran. Najran has oases, mountains, and desert at its eastside. The average temperature ranges from 14.6 to 30.9 ºC. The average annual rainfall is 83-mm.It is southwestern Saudi Arabia near the border with Yemen. It is the capital of Najran Province(Figure 1).

Moral sagacity: The university ethical board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board.

Sample collection and transport: Vaginal and cervical swabs were collected from aborted mares in sterilized tube containing sterilized nutrient broth and transported into the Microbiology laboratory in the Department of Applied Medical Sciences, Community College, Najran University within an hour of collection.

***Culture and identification*:** The samples in nutrient broth were incubated 37 ºC for 24 hours.Culturing from each tubewere streaked on blood agarandMcConkey agar plates and incubated at 37ºC for 24 hours. Isolated purecolonies were subcultured on nutrient agar plates. Pure colonies were identified by biochemical method, the Microbact™ Gram-negative system (Oxoid, UK)which used for the identification of aerobic and facultatively anaerobic Gram-negative bacteria (Enterobacteriaceae and miscellaneous Gram- negative bacteria) (Mugg, 1981). The positive specimens were then subcultured in nutrient broth and stored in the refrigerator at 8ºC for Antimicrobial susceptibility testing.

***Antimicrobial susceptibility testing*:** Antimicrobial susceptibility tests were performed on Mueller-Hinton agar (Oxoid, Hampshire, UK) by disc diffusion method (Bauver*et al*., 1966). The antimicrobial agents tested were: Sulfamethoxazole and Trimethoprim (SXT) (25 µg), Ciprofloxacin (CIP) (5 µg), Gentamicin (CN) (10 µg), Ceftriaxone (CRO) (30 µg), Amoxicillin &Clavulinic acid (AMC) (30 µg), Norfloxacillin (NOR) (10 µg), Cephradine (CE) (30 µg), Ampicillin (AMP) (10 µg) and Tetracycline (TE)(10 µg) (Oxoid, UK). The resistance and sensitivity were interpreted according to the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1993).

Figure 1. Map of Saudi Arabia showing the geographic location of Najran (arrow), region of Saudi Arabia, located in the south of the country along the border with Yemen

RESULTS

Examination of the equine farms in El forresia club revealed bad hygiene and had no Veterinary care. According owner history, the age of mares was ranged from five to ten years old. Most of abortions were in last trimester of pregnancy. The foetuses were born dead. Abortion rate was 5.9 % (5/85). Isolation and identification of *Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumoniae*from aborted mareswere depended on the characters and features of colonies of *Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumoniae*on blood agar plate and McConkey agar plates in addition to biochemical tests biochemical tests to isolated colonies from aborted cases according to Microbact™ Gram-negative system (Table 1,2).

Five mares have been aborted. Pure colonies of *Enterobacteragglomerans (Pantoeaagglomerans)* were isolated from two mares while Pure colonies of *Klebsiella pneumonia* were isolated from one mare. Mixed infection of *Enterobacteragglomerans (Pantoeaagglomerans), Klebsiellapneumoniae, Escherichia coli and Citrobacter spp.* was isolated from two-aborted mare. On blood agar plate, the *Enterobacteragglomerans* was yellow-pigmented colonies, non-haemolytic, 1-2 mm in diameter and convex while on McConkey agar plates, it was translucent, smooth and pale yellow reddish. *Klebsiellapneumoniae* was yieldedmucoid, large and white grey colonies on blood agar plate and pink mucoid colonies on McConkey agar plates. Isolatedcolonies of *Enterobacteragglomerans (Pantoeaagglomerans)* were susceptible to Ciprofloxacin (CIP) (100%) with inhibition zone ranged from 25-30 mm in diameter. It was susceptible toNorfloxacillin (NOR) (75%) with inhibition zone ranged from 15-27 mm mm in diameter.

Table 1. The result of biochemical tests to isolated colonies from aborted cases according to Microbact™ Gram-negative system

Biochemical tests		Isolated colonies from aborted cases	
		E. agglomerans	K. pneumonia
LYS	Lysine Decarboxylase	Negative	Positive
GLU	Acid from Glucose	Positive	Positive
ONP	ONPG	Negative	Positive
VP	VogesProskauer	Positive	Positive
GEL	Gelatin Liquefaction	Negative	Negative
SOR	Acid from Sorbitol	Positive	Positive
LAC	Acid from Lactose	Positive	Positive
RAF	Acid from Raffinose	Negative	Negative
ORN	Ornithine Decarboxyl	Negative	Negative
MAN	Acid from Mannitol	Negative	Positive
IND	Indole	Negative	Negative
CIT	Citrate Utilization	Positive	Positive
MAL	Malonate Inhibition	Negative	Negative
RHA	Acid from Ramnose	Positive	Positive
ARA	Acid from Salicin	Positive	Positive
SAL	Acid from Salicin	Negative	Negative
H2S	H2S Production	Negative	Negative
XYL	Acid from Xylose	Negative	Positive
UR	Urea Hydrolysis	Negative	Positive
TDA	Tryptophan Deaminase	Negative	Negative
INO	Acid from Inositol	Negative	Positive
SUC	Acid from Sucrose	Positive	Positive
ADO	Acid from Adonitol	Negative	Positive
ARG	Arginine Dihydrolase	Negative	Negative

Table 2. The identification to isolated colonies from aborted cases according to Microbact™ Gram-negative system

	Identification methods to isolated colonies of K. pneumonia	Result	Identification methods to isolated colonies of E. agglomerans	Result
1-	Probability	1/206,017<	Probability	1/100,000,000<
2-	Percent Probability	99.79%	Percent Probability	96.60%
3-	1st Test Against RAF	99.0%	1st Test Against MAN	99.9%
4-	2nd Test Against SAL	99.0%	2nd Test Against XYL	93.0%
5-	3rd Test Against MAL	93.0%	3rd Test Against ONP	90.0%
6-	Additional tests	Yes	Additional tests	Yes
7-	Motility (37 °C)	0.1%	DNase (25 °C)	0.1%
8-	DNase (25 °C)	0.1%	Motility (37 °C)	85.0%
9-	Alpha Methyl D Gluc	90.0%	Acid from Cellobiose	55.0%
10-	Methyl red	10.0%	Esculin Hydrolysis	60.0%
11-	Acid from Arabitol	98.0%	Acid from Melibiose	50.0%
12-	KCN Inhibition	98.0%	Gas from D- Glucose	20.0%
13-	Preferred ID Choice	K. pneumonia	Preferred ID Choice	E. agglomerans

Table 3. Antimicrobial susceptibility patterns of *E. agglomerans* isolated colonies from aborted cases

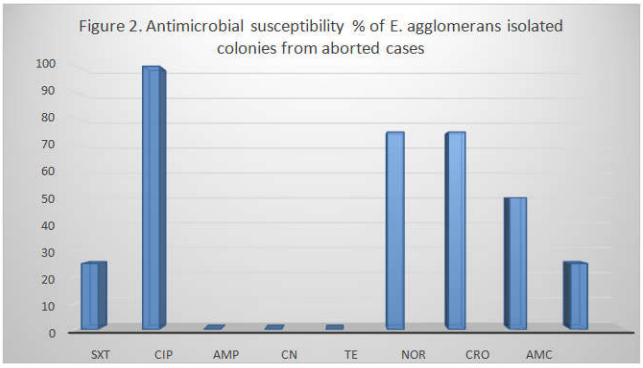
Antibiotic disc concentration	Antimicrobial susceptibility patterns of isolated colonies from aborted cases		
	Disc conc.	Inhibition Zone Diameter range in mm	Susceptible %
Sulfamethoxazole and Trimethoprim (SXT)	25 µg	15-26 mm	25%
Ciprofloxacin (CIP)	5 µg	25-30 mm	100%
Ampicillin (AMP)	10 µg	6-10 mm	0%
Gentamicin (CN)	10 µg	10-17 mm	0%
Tetracycline (TE)	10 µg	20-22 mm	0%
Norfloxacillin (NOR)	10 µg	15-27 mm	75%
Ceftriaxone (CRO)	30 µg	12- 25 mm	75%
Amoxicillin &Clavulinic acid (AMC)	30 µg	10-22 mm	50%
Cephradine (CE)	30 µg	8-20 mm	25%

Table 4. Antimicrobial susceptibility patterns of *Klebsiellapneumoniae*isolated colonies from aborted cases

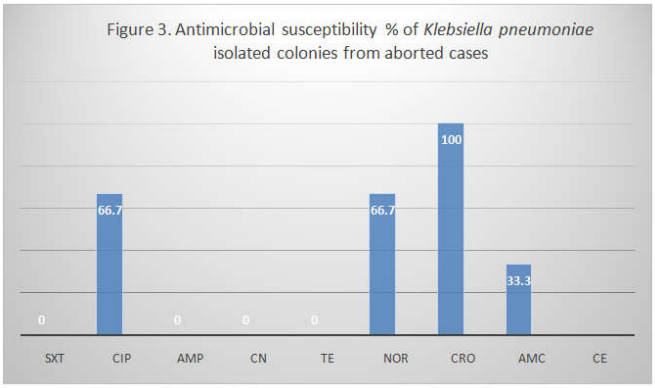
Antibiotic disc concentration	Antimicrobial susceptibility patterns of isolated colonies from aborted cases		
	Disc conc.	Inhibition Zone Diameter range in mm	Susceptible %
Sulfamethoxazole and Trimethoprim (SXT)	25 µg	11-14 mm	0%
Ciprofloxacin (CIP)	5 µg	13-26 mm	66.7%
Ampicillin (AMP)	10 µg	7-11 mm	0%
Gentamicin (CN)	10 µg	7-15 mm	0%
Tetracycline (TE)	10 µg	17-20 mm	0%
Norfloxacillin (NOR)	10 µg	13-23 mm	66.7%
Ceftriaxone (CRO)	30 µg	25-30 mm	100%
Amoxicillin &Clavulinic acid (AMC)	30 µg	16-18 mm	33.3 %
Cephradine (CE)	30 µg	10-22mm	33.3%

It was susceptible to Ceftriaxone (CRO) (75%) with inhibition zone ranged from 12-25 mm in diameter. It was susceptible to Amoxicillin &Clavulinic acid (AMC) (50%) with inhibition zone ranged from 10-22 mm in diameter. It was susceptible to Sulfamethoxazole and Trimethoprim (SXT) (25%) with inhibition zone ranged from 15-26 mm in diameterandCephradine (CE) (25%) with inhibition zone ranged from 8-20 mm in diameter. while they were resistant to Ampicillin (AMP), Gentamicin (CN) and Tetracycline (TE) (Table 3 and figure 2).

Furthermore, Isolated colonies of *Klebsiella pneumonia* were susceptible to Ceftriaxone (CRO) (100%) with inhibition zone ranged from 25-30 mm in diameter. It was susceptible to Ciprofloxacin (CIP) (66.6%) with inhibition zone ranged from 13-26 mm mm in diameter.It was susceptible to Norfloxacillin (NOR) (66.6%) with inhibition zone ranged from 13-23 mm in diameter. It was susceptible to Amoxicillin &Clavulinic acid (AMC) (33.3%) with inhibition zone ranged from 16-18 mm in diameter and Cephradine (CE) (33.3%) with inhibition zone ranged from 10-22 mm in diameter while they were resistant to Ampicillin (AMP), Gentamicin (CN), Tetracycline (TE), Sulfamethoxazole, and Trimethoprim (SXT) (Table 4 and Figure 3).



Sulfamethoxazole and Trimethoprim (SXT) (25 µg), Ciprofloxacin (CIP) (5 µg), Gentamicin (CN) (10 µg), Ceftriaxone (CRO) (30 µg), Amoxicillin &Clavulinic acid (AMC) (30 µg), Norfloxacillin (NOR) (10 µg), Cephradine (CE) (30 µg), Ampicillin (AMP) (10 µg) and Tetracycline (TE) (10 µg)



Sulfamethoxazole and Trimethoprim (SXT) (25 µg), Ciprofloxacin (CIP) (5 µg), Gentamicin (CN) (10 µg), Ceftriaxone (CRO) (30 µg), Amoxicillin &Clavulinic acid (AMC) (30 µg), Norfloxacillin (NOR) (10 µg), Cephradine (CE) (30 µg), Ampicillin (AMP) (10 µg) and Tetracycline (TE) (10 µg)

DISCUSSION

The major problems of equine breeding in all farms are the infertility,stillbirth and abortions. These problemsare usually caused by infectious agent (Singh, 2003 and Singh, 2014). In spite of the fact thatmany microbes have been associated with abortion and infertility in equine breeding, including of Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumoniae with these diseases has rarely been reported.In this present study, the *E. agglomerans* and *Klebsiellapneumoniae* mare abortion has been recorded for first time in southeren area Najran region Saudi Arabia. In addition to, *E. agglomerans* has been isolated from fetal fluid and abortion in mares for first report as systematic investigation (Singh, 2003 and Malik, 2002). In france, abortion in mares due to *E. agglomerans* is not uncommon with 4.9-11.5% therefore, *E. agglomerans* mare abortion is recorded in temperate zone (Fontaine *et al.,* 1993) In our study all isolates from mares abortion were resistant to Ampicillin (AMP), Gentamicin (CN), Tetracycline (TE) antibiotic disc. it has been similar study revealed that all isolates of *Enterobacteragglomerans, Pseudomonas aeruginosa, Citrobacterfreundii*and *Escherichia coli*from abortion in mares were resistant to ampliclin andstreptomycin (Singh, 2004). In the present study, mixed infection of *Enterobacteragglomerans (Pantoeaagglomerans), Klebsiellapneumoniae, Escherichia coli and Citrobacter spp.*was isolated from two-aborted mare. This mixed infection isolated from vaginal swab was due to secondary bacteria invasion. Besides, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus spp., Klebsiella pneumoniae, Enterobacteragglomerans, Taylorellaequigenitalis (CEM), Streptococcus equisimilis, Leptospirapomona, Corynebacteriumequi, Brucellaabortus, Actinobaccillus spp*. and *Rhodococcusequi* may also cause abortion in the pregnant mare either alone or in concert with other pathogens (Singh, 2003; Singh, 2004).In Italy isolation of five cases of abortion in mares have been caused by*Klebsiellapneumoniae* septicaemia and one case of abortion due to placentitiscaused by *Klebsiellapneumoniae*(Marenzoni, 2012).Antibiotic drug resistance present in those strains reveals prevalence of bacteria multiple drug resistantat the farm those may become problem at any time in future by acquiring needful virulence. All thebacteria isolated in the study belong to those cause abortions in mares however, their prevalence vary from place toplace (Fontaine, 1993 and Garg, 1986).

Conclusion

Strict protocols of good practices should beassured in order to prevent contamination and transmission of microbes. This shouldinclude continuous education on good quality hand hygiene andrigorous observation of environment control in the handlingarea of equine farm. The isolation of *Enterobacteragglomerans (Pantoeaagglomerans) and Klebsiellapneumonia*from vaginal cervico swabs after mare's abortion suggests them to be a cause of abortion in mares.

Acknowledgements

The author thankssincerely theadministrator and officials ofequinefarmsin El forresiaclub,Najran, Saudi Arabia and Microbiology laboratory in the Department of Applied Medical Sciences, Community College, NajranUniversityfor facilitating the performance of this research and helping the sampling for investigation.

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