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Research Article

DRUG RESISTANCE STRANGLES IN PARASITIZED (GASTROINTESTINAL PARASITES) HORSES

***[1,2]Mosa Mohamed Bahnass**

[1]Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Zagazig University, Egypt
[2]Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi

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ABSTRACT

The hazards of Strangles and Gastrointestinal parasites is major problems in equine farms.They cause losses and complications among ponies and aged horse. This study was concerned with the prevalence of atypical mild Strangles, GIT parasites, significant EPG and antimicrobial resistance Streptococcus equi subsp. equi. In equine farm. Nasal swab and faecal samples were collected from six stables in Horse farm (N = 37 horses) at summer's season 2015. Isolation and identification of S. equi colonies were done according to culturing characters, Gram's staining, biochemical methods and Lancefield grouping. Faecal examination was applied by Sedimental-centrifugal flotation method to identify GIT eggs. The faecal egg counts (EPG) were performed using amodifiedMcMaster technique. The overall prevalence of strangles was 70.3 % (26/37). Atypical Mild non-cured drug resistance Strangles prevalence were 23 % (6/26).The overall prevalence of GIT parasite was 45.9 % (17/37) in all horse stables.GIT parasites cases (EPG ≥ 200) were 58.8% (10/17). Isolated *Streptococcus equi* subsp. *equi*were significantly higher sensitive to Ciprofloxacin (5µg) (100 %). Isolated colonies were completely resistant to Sulfamethoxazole and Trimethoprim (SXT), Ampicillin (AMP), Gentamicin (CN), Ceftriaxone (CRO), Amoxicillin &Clavulinic acid (AMC) and Cephradine (CE). In conclusion, GIT parasites was significantly higher in young and old horses. Furthermore, atypical mild drug resistance Strangles case significantly higher in GIT parasites case (EPG ≥ 200).

INTRODUCTION

Strangles is bacterial contagious upper respiratory tract infection of Equinesspecially the horses caused by Gram positive *Streptococcus equi var. equi*. It belongs to the pyogenic group of streptococci (group C of the Lancefield classification) (Marchandin *et al*., 2007). The causative agent consists of three subspecies of zoonotic agents rarely reported as human pathogens: *S. equi subsp. equi, S. equi subsp. zooepidemicus* and *S. equi subsp. ruminatorum*. After 4-8 days (incubation period), the affected animals has fever, bilateral mucopurulent nasal discharge and swollen or enlarged lymph nodes in the neck and throat. They may also stop eating and have a dull aspect. Atypical strangles is now commonly recognised whereby infected horses display minor or no clinical signs (Sweeney*et al*., 2005). The infected horse may exhibit a mild respiratory infection but no abscesses develop. As a result, Strangles can be overlooked as the symptoms displayed are commonclinical signs for other diseases (Czernomysy *et al*., 2010). Method of collection samples is various to detect S. equi, including use of nasal swabs, nasopharyngeal swabs (Newton*et al*., 1997), nasopharyngeal lavages (Laus *et al*., 2007)or even lavages from the guttural pouches (Newton *et al*., 2000). Gastrointestinal parasite is of health hazard to equine. There are sixty species of Gastrointestinal parasite in horses and may contain more than one species in the same time (Stoltenow and Purdy 2006). Internal Helminths in equine cause morbidity and mortality and the prevalence is usually high enough to clinical parasitism (Proudman and Mattews 2000). In Saudi Arabia, equine Parastic study was very restricted in external parasites (Al-Khalifa *et al*., 1983). Whereas first Helminthic study in Saudi Arabia was Prevalence of non-strongyle gastrointestinal parasites of horses in Riyadh region of Saudi Arabia Saudi (Anazi *et al*., 2011) *Streptococcus equi var. equi* has been found to be susceptible to trimethoprim/sulfamethoxazole antibiotic where as 98% of S. equi strains are sensitive to erythromycin and clindamycin (Meyer *et a* findings wereperformed to isolation, identification and *l*., 2011 and Minces *et al*., 2011). The present investigation was to detect antimicrobial susceptibility pattern of *Streptococcus equi*

Corresponding author: Mosa Mohamed Bahnass,
[1]Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Zagazig University, Egypt.
[2]Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi.

subsp. *equi*strains in atypical mild drug resistance Strangles cases. Moreover, Faecal examination and EPG was doneto equine farm specially theatypicalmild cases of Strangles.

MATERIAL AND METHODS

The owner complain and history: The present study has been applied during summer season 2015 in equine farms tables (37 horse) located in Najran region, Saudi Arabia. The signs in horses between 1-35 years old were started with high fever about 39.2 ºC, loss of appetite, depression, lethargy, purulent nasal discharge bilateral, enlargement of mandibular and retropharyngeal lymphnodes with cardinal signs of inflammation. Penicillin antibiotic, antipyretic and anti-inflammatory have been used in all cases (26 cases). All cases have been cured except six cases. These cases had less bilateral mucoid nasal discharge and less degree of appetite and the head lymph nodes were slightly enlarged.

Current investigation region: The current investigation geographic area was Najran.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.

Collection of the samples and its transport: Nasal swabs have been collected from six non cured cases in sterilized tube containing sterilized nutrient broth. Faecal samples were collected from all horses in the farm. The nasal swabs and faecal samples have been transported in two clean containers to the Microbiology laboratory in the Department of Applied Medical Sciences, Community College, Najran University within an hour of collection.

Faecal examination: The faecal samples were collected at different times of the day. The samples were examined by the Sedimental-centrifugal flotation (Martins *et al*. 2003). The faecal egg counts were performed using amodified McMaster technique with a50 eggs per gram sensitivity (Roepstorff and Nansen, 1998). The cut-off value for significant count was 200 EPG (Egg Per Gram), which is used by a majority of equine practices Danish (Nielsen *et al*., 2005). Also itcorresponds with the 0–500 EPG range of cut-offvalues reported by Uhlinger (1993). One faecal sample was collected and analysed from each horse at eachoccasion during the study.

Culture and identification: Nasal swab samples in nutrient broth were incubated 37 ºC for 24 hours. Culturing from each tube were streaked on blood agar (5% sheep blood) plates and incubated at 37ºC for 24 hours then the isolated culture were identified by biochemical methods (Quinn *et al*., 1994), Gram's staining and Lancefield grouping according to StrepPRO™ Grouping Kit (The Hardy Diagnostics, 1430 West McCoy Lane, Santa Maria, CA 93455, USA). Lancefield grouping isa latex serological test which relies on the detection of group antigen on the bacterial surface (if the result shows a C group of Streptococci. Isolated pure colonies were subcultured on nutrient agar plates. 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, 1993).

Statistical analysis

Data were performed using SPSS (Statistical Package forSocial Sciences) (16.0, 2008) for windows software and for subsequent calculations.The datawas compared using the subprogram Post Hoc Test, One-way'ANOVA' with the F-statistic for a test of significant P value($p< 0.05$).

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

RESULTS

In the equine farm, there were six horsesst ables.From the history of the owner, the overall prevalence of strangles was 70.3 % (26/37). The highest prevalence was in stable one 80% (4/5) while the lowest prevalence was in stable three 55.6 % (5/9). The signs of atypical lmild drug resistance Strangles cases were slight bilateral muco-purulent nasal discharge and slight submandibular lymphadenopathy (Figure 4). Atypical mild non-cured drug resistance Strangles cases prevalence were 23 % (6/26). The highest prevalence of atypical drug resistance Strangles cases was in stable six 66.7% (2/3) while the prevalence in stables (1, 2, 3, 4 and 5) was 25 %, 40%, 20 %, 0 % and 0% respectively(table 1 and figure 2). According to biochemical tests, Gram's staining and Lancefield grouping, the isolated colonies were identified as *Streptococcus equi* subsp. *equi* which the causative microbe of Strangles. Small transluscent colonies on blood (5% sheep blood) agar after incubation for 24 h at 37°C. Cleared Beta-hemolysishas been occurred on blood agar. Lancefield group C was yielded.

Table 1. Cases of Strangles in horse farm Stables

Horse stables	No. of Horses	Age range	Strangle cases		Atypicalmild Strangles cases	
			No.	%	No.	%
Stable 1	5	1-4	4	80	1	25
Stable 2	7	1-2	5	71.4	2	40
Stable 3	9	3-35	5	55.6	1	20
Stable 4	7	1-3	4	57.1	0	0
Stable 5	5	1-2	3	60	0	0
Stable 6	4	One year	3	75	2	66.7
Total	37	1-35	26	70.3	6	23

Table 2. Antimicrobial susceptibility patterns of *Streptococcus equi* subsp. *equi*solated colonies from atypicalmild Strangles 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	22-26 mm	100%
Ampicillin (AMP)	10 µg	7-11 mm	0%
Gentamicin (CN)	10 µg	5-15 mm	0%
Tetracycline (TE)	10 µg	17-20 mm	33.3 %
Norfloxacillin (NOR)	10 µg	13-23 mm	50 %
Ceftriaxone (CRO)	30 µg	7-10 mm	0 %
Amoxicillin &Clavulinic acid (AMC)	30 µg	3-5 mm	0 %
Cephradine (CE)	30 µg	8-11 mm	0 %

Table 3. Cases of Gastrointestinal parasites in horse farm Stables

Horse Stables	No. of Horses	Gastrointestinal parasites						Significant EPG ≥200					
		Ascaris	%	GIT	%	Total	%	Ascaris	%	GIT	%	Total	%
Stable 1	5	1	20	2	40	3	60	1	33.3	0	0	1	33.3
Stable 2	7	0	0	2	28.6	2	28.6	0	0	2	100	2	100
Stable 3	9	0	0	1	11.1	1	11.1	0	0	1	100	1	100
Stable 4	7	0	0	2	28.6	2	28.6	0	0	1	50	1	50
Stable 5	5	2	40	3	60	5	100	1	20	0	0	1	20
Stable 6	4	4	100	0	0	4	100	4	100	0	0	4	100
Total	37	7	18.9	10	27	17	45.9	6	35.3	4	23.5	10	58.8

EPG: Egg Per Gram of feces, GIT: Gastrointestinal Nematodes.ST: Strangles %, CST: Chronis Strangles%, Sig. EPG: Significant Egg Per Gram of feces, GIT: Gastrointestinal Nematodes.

Antimicrobial susceptibility patterns of *Streptococcus equi* subsp. *equi* of isolated colonies fromatypicalmild Strangles cases was applied (Table 2 and Figure 3). Isolated colonies were susceptible to Ciprofloxacin (CIP), Norfloxacillin (NOR) and Tetracycline (TE) with pattern of 100 %, 50 % and 33.3 % respectively.

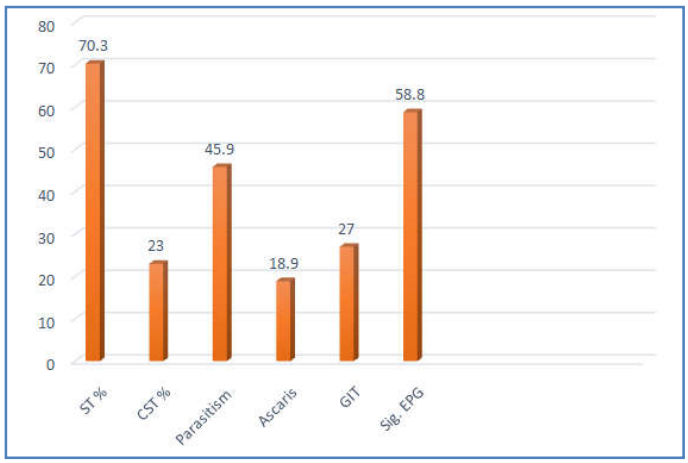
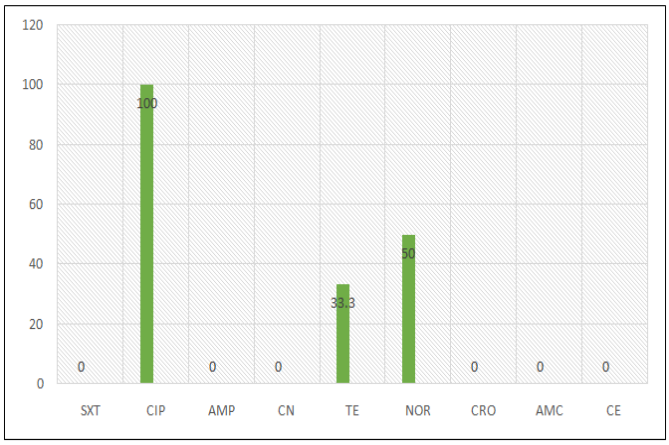


Figure 2. Cases of Strangles and Gastrointestinal parasites in horse farm Stables

On the other hand, Isolated colonies were completely resistant to Sulfamethoxazole and Trimethoprim (SXT), Ampicillin (AMP), Gentamicin (CN), Ceftriaxone (CRO), Amoxicillin & Clavulinic acid (AMC) and Cephradine (CE) with pattern of 0% (Table 2 and Figure 3). From antitimicrobial susceptibility patterns of *Streptococcus equi* subsp. *equi* of isolated colonies revealed that atypical mild non-cured drug resistance Strangles was highly susceptible to treatment with Ciprofloxacin (CIP) antibiotic. Isolated *Streptococcus equi* subsp. *equi* were significantly higher sensitive ($P<0.05$) to Ciprofloxacin (5µg) (100 %). Faecal examination and EPG wasdone to of equine farm especially atypical mild cases of Strangles. Ascaris and GIT Nematodes egg were detected in faecal examination (table 3 and figure 5,6). The overall prevalence of GIT parasite was 45.9 % (17/37) in all horse stables. Whereas, the prevalence of Ascariasis and GIT Nematodes were 18.9 % (7/37) and 27 % respectively. The cases of GIT parasites were significant to be treated with anthelmintic at EPG ≥ 200. GIT parasites cases (EPG ≥ 200) were 58.8% (10/17). Ascaris and GIT Nematodes egg (EPG ≥ 200) were 35.3% (6/17) and 23.5% (4/17) respectively. GIT parasites was significantly higher ($P<0.05$) in young and old horses. Furthermore, atypical Mild non-cured drug resistance Strangles were in significantly higher ($P<0.05$) in GIT parasites cases.



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)

Figure 3. Antimicrobial susceptibility % of Streptococcus equi subsp. equi isolated colonies from mild Strangles case

Figure 4. Male horse with sign of atypicalmild Strangles, slight bilateral mucoid nasal discharge and slight submandibular lymphadenopathy

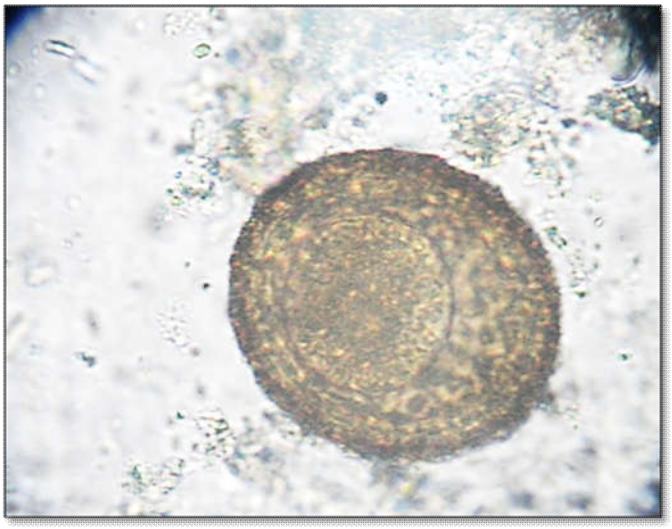


Figure 5. Ascaris egg from horse feces

Figure 6. Gastro Intestinal Nematodes (GIT) egg from horse feces

DISCUSSION

Strangles is equine, contagious, world wide and upper respiratory bacterial disease of high morbidity and low motility (Sweeney *et al*., 2005; Duffee *et al*. 2015). Internal parasites are common in horses even in highly effective broad-spectrum anthelmintic drugs (Edmonds *et al*., 2001). The cases of GIT parasites were significant to be treated with anthelmintic at EPG $\geq$ 200. The faecal egg counts were performed using amodified McMaster technique with a 50 eggs per gram sensitivity (Roepstorff and Nansen, 1998). The cut-off value for significant count was 200 EPG (Egg Per Gram), which is used by a majority of equine practices Danish (Nielsen *et al*., 2005). Also it corresponds with the 0–500 EPG range of cut-offvalues reported by Uhlinger (1993). One faecal sample was collected and analysed from each horse at each occasion during the study. In present study, nasal swab was used only to isolation of Streptococcus equi in culture. Performing of PCR real-time directly on nasopharyngeal lavage and a single additional sample such as a nasal swab (ESwab) appears to be equally effective in recovery of S. equi to confirm a diagnosis of strangles, but without the time delay imposed by culture (Lindahl *et al*., 2013). In the present investigation, GIT parasites was significantly higher ($P<0.05$) in young and old horses. Furthermore, atypical Mild non-cured drug resistance Strangles were in significantly higher ($P<0.05$) in GIT parasites cases. The relation between the highly parasitism and atypical mild non-cured drug resistance Strangles horses would have likely resulted from themiss used antibiotic or bad immunity.These default consistent withthat heavily parasitized ponies have uniformly decreased cellular and humoral immune responses to soluble protein immunisation. The mechanisms involved may have potential deleterious effects on standard vaccine protocols of parasitized equines (Edmonds *et al*., 2001). In approximately 10 percent of cases infected horses become carriers even though they themselves have recovered and appear healthy. Carriers have become persistently infected when abscess material forms in the guttural pouches (air sacs in the back of the throat) of the horse and is not completely eliminated. The bacteria can survive in the guttural pouchfor

months or even years. Carriers can therefore continue to spread the disease causing new outbreaks if the horse becomes stressed, for instance moving to a new yard (Sweeney*et al*., 2005). There is proof that trimethoprim-sulfadiazine did not eliminate *S zooepidemicus* infection in subcutaneously implanted tissue chambers in ponies, the study did not define its efficacy against *S equi.* (Ensink *et al*., 2003). Resistance incidence to most other antibiotics is low with the exception of aminoglycoside resistance, including gentamicin, which isconsistently observed (Sweeney *et al*., 2005). in contrast, the present study was investigated that on the other hand, Isolated colonies were completely resistant to Sulfamethoxazole and Trimethoprim (SXT), Ampicillin (AMP), Gentamicin (CN), Ceftriaxone (CRO), Amoxicillin &Clavulinic acid (AMC) and Cephradine (CE).The main parasites groups affecting equids are the ascarids, largestrongyles, small strongyles (GIT Nematodes) and tapeworms however although, species of protozoan are also embroiled (Nielsen *et al*., 2014).

Conclusion

Equine GIT parasites are a risk and predisposing factors to viral and bacterial diseases outbreaks in equine that they affect on immunity therefore, Our findings suggests that strict hygiene, strategic and tactical control measure to Internal GIT parasites should be applied in equine farm. Anthelmintic drug must be applied in constant interval and perfect dose. Avoiding sub-doses to antibiotic in Strangles cases is important requirement to avoid antimicrobial resistance. Atypical mild Strangles play a role in drug resistance and carrier that can therefore continue to spread the disease causing new outbreaks if the horse becomes stressed, for instance moving to a new yardVeterinarians must be conversant with all strangles information in devising control procedures and in the management of strangles outbreaks.

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