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Tetanus in horses: an overview of 70 cases[1]

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ABSTRACT.- Ribeiro M.G., Nardi Júnior G., Megid J., Franco M.M.J., Guerra S.T., Portilho F.V.R., Rodrigues S.A. & Paes A.C. 2018. **Tetanus in horses: an overview of 70 cases.** *Pesquisa Veterinária Brasileira 38(2):285-293*. Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Cx. Postal 560, Botucatu, SP18618-681, Brazil. E-mail: mgribeiro@fmvz.unesp.br

Tetanus is characterized by high case fatality rates in horses. Comprehensive case series studies involving equine tetanus from different geographic areas enable the evaluation of prognosis, efficacy of treatment, and control measures. We retrospectively investigated some selected epidemiological data (breed, age, gender, use of the horses, history of vaccination, seasonality, presence of wound/history of surgical procedures, clinical outcomes) and main clinical aspects (clinical signs, incubation period, length of hospitalization, and period between onset signs and hospitalization) in 70 cases of equine tetanus over 1990-2015, with emphasis in the association between these data and the clinical outcomes. High mortality rate (72.9%) was observed in this study. Forty (57.1%) horses presented history of wounds or surgical procedures related with tetanus, represented mainly by lesions in the hind limbs (42.5%), front limbs (15.0%), umbilical infections (7.1%), castration (4.3%), and face wounds (4.3%). Hyperesthesia, limb spasticity, cervical stiffness, tetanic spasms, and restriction of jaw movement were the main consistent clinical signs. Besides no statistical association, all the horses with umbilical infections, wounds in face, prolonged recumbency, sweating, dysphagia/aphagia died, and together with delay between onset of first clinical signs and prompt veterinary assistance (< 5 days) were considered indicative of poor prognosis; whereas there was a significant association (p=0.001) between survival and length of hospitalization > 7 days, seemed as an evidence of good prognosis. The high mortality rate of tetanus, even in horses under specific treatment, highlight the need for early diagnosis, prompt veterinary assistance, and establishment of prophylatic measures in equine farms.

INDEX TERMS: Tetanus, *Clostridium tetani*, equine, epidemiological data, clinical signs, bacterioses.

RESUMO.- [**Tétano em equinos: um panorama de 70 casos.**] Tétano é caracterizado por elevada mortalidade em equinos. Estudos envolvendo séries de casos da doença provenientes de diferentes áreas geográficas possibilitam avaliar o prognóstico, eficácia do tratamento e ações de controle. Foram avaliados, retrospectivamente, os principais achados epidemiológicos (raça, idade, sexo, uso dos equinos, história de vacinação, sazonalidade, presença de lesões/histórico de procedimentos cirúrgicos e evolução dos casos) e aspectos clínicos (sinais clínicos, período de incubação, tempo de hospitalização e período entre início dos sinais e hospitalização) em 70 casos de tétano em equinos atendidos entre 1990 e 2015, com ênfase na associação entre os dados clínico-epidemiológicos e a evolução dos casos (mortalidade). Foi observada alta mortalidade (72,9%) nos equinos. Do total de equinos, 57,1% apresentavam lesões ou histórico de procedimentos cirúrgicos relacionados com tétano, representados principalmente por lesões em membros posteriores (42,5%), membros anteriores

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(15,0%), infecções umbilicais (7,1%), castração (4,3%) e lesões de face (4,3%). Hiperestesia, espasticidade dos membros, rigidez de pescoço, espasmos tetânicos e restrição aos movimentos de mandíbula foram os principais sinais clínicos observados. Apesar da ausência de significância estatística, todos os animais com presença de infecções umbilicais, lesões na face, decúbito prolongado, sudorese e disfagia/afagia evoluíram para óbito e, associado ao início dos sinais clínicos e a demora no atendimento (< 5 dias), foram considerados indicativos de prognóstico reservado. Em contraste, foi observada associação significante (p=0,001) entre a sobrevivência dos animais e o tempo de hospitalização maior que 7 dias, considerado uma indicação de bom prognóstico. A elevada mortalidade do tétano, mesmo em equinos tratados, enfatiza a necessidade do diagnóstico precoce, do rápido atendimento veterinário e adoção de medidas profiláticas nos criatórios.

TERMOS DE INDEXAÇÃO: Tétano, *Clostridium tetani*, equinos, epidemiologia, sinais clínicos, bacterioses.

INTRODUCTION

Tetanus is an ancient non-contagious infectious disease caused by exotoxins produced by *Clostridium tetani*, affecting domestic animals, wildlife, and humans. Despite the centenary knowledge on causal agent, serum antitoxin, and tetanus toxoid (Bleck 2005), the disease remains lethal among livestock. Horses are consistently reported as the most susceptible domestic species to the effects of tetanus toxin (Constable et al. 2016). *C. tetani* is a well-known Gram-positive spore forming anaerobic bacterium (Quinn et al. 2011). This neurotoxic clostridium is a soil inhabitant and can be found in the feces of livestock, particularly horses. Equine tetanus is intimately associated with intensively farmed areas. Warm temperatures and humidity, as well as neutral pH of soil are favorable conditions to organism that increase the prevalence of the disease in tropical countries (Mackay 2014).

Case fatality rates for equine tetanus are widely variable among countries and regions. Despite the decrease in occurrence of disease with the advent of vaccination, equine tetanus remains associated with high fatality rates, especially in developing countries where vaccination is not a usual prophylactic measure. In this scenario, most retrospective studies involving equine tetanus refer mortality rates ranging about 50% to 80% of animals (Kay & Knottenbelt 2007, Reichmann et al. 2008, Gračner et al. 2015).

Equine tetanus is described as case reports (Mykkänen et al. 2011) or outbreaks worldwide (Constable et al. 2016). Only a few comprehensive case series studies have been reported (Mackay 2014), which may to enable the recognition of the main epidemiological and clinical aspects of tetanus (Kay & Knottenbelt 2007), the evaluation of prognosis (Reichmann et al. 2008), the efficacy of therapy (Gračner et al. 2015), and the control measures recommended for the disease (Green et al. 1994). The aim of the present study was retrospectively to investigate some selected epidemiological and clinical aspects in 70 cases of equine tetanus during a twenty-six-year period, with emphasis in the association between these data and the clinical outcomes.

MATERIALS AND METHODS

A cross-sectional study of the main epidemiological data and clinical findings in 70 cases of equine tetanus recorded in the Infectious Diseases of Domestic Animals Service at Veterinary Hospital of the São Paulo State University-Unesp, Botucatu, SP, Brazil, over 1990-2015, was retrospectively investigated.

Criteria for case selection and database of study. All cases of equine tetanus admitted at Veterinary Hospital over 26-year period were included in the study, and animals came from the state of São Paulo, Brazil, where horse breeding is common. The database of study was exclusively paper records analyzed manually.

Epidemiological and clinical data. The major parameter data comparison was clinical outcome (number of surviving and mortality) versus selected epidemiological and clinical data. The effect of mortality (yes/no) of disease was assessed using following selected data: age, gender (male/female), breed, use of the horses, history of vaccination (yes/no), presence of wound or history of surgical procedures (castration, umbilical infection, shoeing, recent injections) (yes/no), and occurrence of cases in the spring (September to November), summer (December to February), autumn (March to May), and winter (June to August). Diagnosis was based on data in the medical records and clinical examination of each horse (Green et al. 1994, Kay & Knottenbelt 2007, Reichmann et al. 2008, Gračner et al. 2015). In addition, incubation period (determined when the time of injury was known), length of hospitalization, and period between first clinical signs and hospitalization were assessed, as well.

Treatment. The treatment protocol of all horses admitted to the Veterinary Hospital was similar to the one recommended elsewhere (Green et al. 1994, Kay & Knottenbelt 2007, Gračner et al. 2015), except in case of individual need for sedation, muscle relaxation, hydration, and maintenance of hydroelectrolytic balance. Benzathine penicillin (40,000 IU/kg, intramuscularly, 5-5 days, 2-3 times) was the antibiotic of choice. Ceftiofur (5-7.5mg/kg, intramuscularly, 24-24 hours, 7-14 duration) was used in recumbent horses. A single dose of tetanus antitoxin (200-250,000 IU, intramuscularly or intravenously) was administrated on the first day of hospitalization. Hydration by intravenous (i.v.) route or via intubation was carried out only in animals that were unable to drink. Isotonic solutions (0.85% NaCl plus 5% glucose) about 6-12% of bodyweight were used daily based on individual needs for hydration, and maintenance of hydroelectrolytic balance, which was evaluated using clinical parameters and/or blood gas parameters (partial pressure of CO_2, partial pressure of O_2, total CO_2 concentration, oxygen saturation, base excess, and concentrations of HCO_3, Na, K, and ionized calcium). Sodium bicarbonate was used intravenously to treat acidosis, if necessary. For sedation and muscle spasm control, acepromazine or diazepam was used (0.05-0.1mg/kg, i.v.). For muscle relaxation, thiocolchicoside was used (4mg per 80-100kg bodyweight, 24-24 hours, 3-5 days). Iodine solutions, hydrogen peroxide, and repellents were used in the topic treatment of wounds. All animals were kept in the hospital in dark and quiet enclosures, with free access to hay and water, at the isolation sector of infectious diseases of livestock in the Veterinary Hospital of Unesp/Botucatu, SP, Brazil. Due to some differences in therapy required due to individual needs for hydration, hydroelectrolytic balance, antimicrobials, and use of sedatives and muscle relaxants,

therapeutic procedures were not considered in further statistical analysis.

Data analysis. The Chi-square (Fisher's exact test) was used to investigate the association between clinical outcomes versus selected epidemiological data, clinical and hospitalization aspects. Statistical analyses were conducted using SPSS version 14 for Windows (SPSS Inc., Chicago, IL, USA), and the level of statistical significance was set at 0.05.

RESULTS

Based on number of horse admissions in the Veterinary Hospital over twenty-six years of study equine tetanus represented 1.21% (n=5,775 horses/70 cases) of the hospital caseload. The selected epidemiological aspects, main clinical signs, hospitalization data, and outcomes among 70 horses with or without wounds or history of surgical procedures related to tetanus are listed in Table 1 and 2.

Mortality. In the current case series, the mortality rates was 72.9% (n=51/70). All the five newborn animals until 30 days of age died (Table 1). In addition, 90% (n=9/10) of animals until 1 year old died (Table 1 and 2).

Age, gender, breed, and use of animals data. Age of the 70 equines ranged from 8 days to 18 years old, with mean age of 5.7±4.2 years (median =5.0 years). From all animals, 51.4% (n=36/70) were mixed breed horses, followed mainly by Mangalarga (n=12/70, 17.2%) and Quarter Horse (n=9/70, 12.9%). There is no information/missing data about breed of 5.7% (n=4/70) horses. Among all horses, 58.6% (n=41/70) were male and 41.4% (n=29/70) female. From the 70 horses, rates of mortality between 0-2 years, 3-4 years, 5-6 years, 7-8 years, 9-10 years, and >10 years were 75.0% (n=12/16), 56.2% (n=9/16), 83.3% (n=10/12), 70.0% (n=7/10), 71.4% (n=5/7), and 88.8% (n=8/9) respectively (Table 1 and 2). No statistical association was found between the clinical outcomes and the age (p=0.59), gender (p=0.31), and breed (p=0.15) of the animals (Table 3). Among all horses, 44.2% were used for farm work (n=31/70), 42.9% sport (n=30/70), and/or 5.8% leisure activities (n=4/70). There is no information/missing data about use of 7.1% (n=5/70) horses. Due to the use of the same horse in two activities, this parameter was not considered in further statistical analysis.

Vaccination. None of the 70 horses analyzed had been vaccinated against tetanus.

Wounds or history of surgical procedures. Among all horses, 57.1% (n=40/70) presented wounds or history of surgical procedures (up until 60 days before) associated with tetanus (Table 1). The main occurrence of this aforementioned data was: wounds in the hind limbs (n=17/40, 42.5%) or front limbs (n=6/40, 15.0%), umbilical infections (n=5/40, 12.5%), wounds in the face (n=4/40, 10.0%), and castration (n=3/40, 7.5%) (Table 1); whereas 42.9% (n=30/70) animals showed no wounds or history of surgical procedures related with disease (Table 2). In the cases with presence of wounds or history of surgical procedures, 67.5% (n=27/40) died (Table 1). Among horses without presence of wounds, 80.0% (n=24/30) died (Table 2). Besides no statistical association (p=0.24) between the outcome and the wounds or history of surgical procedures (Table 3), all the nine horses with umbilical infections or wounds in face died (Table 1).

Seasonality. The occurrence of tetanus among all horses over the spring, summer, autumn, and winter was observed in 25.7% (n=18/70), 17.2% (n=12/70), 21.4% (n=15/70), and 35.7% (n=25/70) animals, respectively (Table 1 and 2). There was no significant association (p=0.86) between the outcome and the occurrence of tetanus in the different seasons (Table 3).

Incubation period. The mean period of time between the identification of wounds or history of surgical procedures related to tetanus and the first clinical sign at veterinary assistance (incubation period) was 19.1±8.5 (median 20.0) days, assessed in 10 animals (range 1-30 days). From these, 6 horses presented incubation period >15 days, from which 5 died. Four horses showed incubation period ≤15 days, from which 3 died (Table 1). There was no association (p=0.67) between the outcome and the incubation period (Table 3).

Clinical signs. Hyperesthesia, limb spasticity, cervical stiffness, tetanic spasms, and restriction of jaw movements were the main signs observed at the first clinical examination of the horses (Table 4). All the 70 animals that were seen at the hospital presented at least four of the aforementioned clinical signs. No clear association was observed between the major clinical signs of tetanus and the clinical outcome, as well as the presence of wounds or history of surgical procedures versus the major clinical signs. All the animals that died (n=51) showed prolonged recumbency, sweating, dysphagia or aphagia. Among all horses, two were admitted to the Veterinary Hospital in decubitus. Due to poor clinical condition this two animals in decubitus were subjected to euthanasia two days after their admission, and were removed from further analysis.

First signs until hospitalization. Among 70 horses, the mean time from the first clinical signs to hospitalization was 3.6 ± 3.9 (median =2.0) days (range 1-20 days). In the fatal outcomes, mean time was 3.0±3.6 (median 2.0) days (range 1-20), whereas in the cases of survivors, it was 5.2±4.4 (median 3.0) days (range 1-22 days). A period >5 days between the first clinical signs and hospitalization was observed in 17.1% (n=12/70) horses, from which 6 died and 6 survived, whereas period < 5 days was reported in 82.9% (n=58/70) horses, from which 45 died and 13 survived. No significant association (p=0.11) between the outcome and the period from the onset of clinical signs to hospitalization was observed (Table 3). However, a tendency toward higher mortality rates was observed among horses with shorter periods (<5 days) from the first clinical signs to hospitalization.

Lenght of hospitalization. Among all horses, 27.1% (19/70) survived after treatment. Among these, 84.2% (n=16/19) horses presented length of hospitalization > 7 days, whereas 15.8% (n=3/19) animals showed length of hospitalization <7 days (Table 1 and 2). There was a significant association (p=0.001) between survival and length of hospitalization >7 days (Table 3). All survivors were released from the Veterinary Hospital when clinical signs solved and after horses were able to ingest food and water normally. No improved survival rate was observed over studied period.

Necropsy findings. All non-survivors were subjected to necropsy. Non-specific gross lesions were observed at necropsy, including pulmonary congestion, edema, and atelectasis, as well umbilical infections and skin wounds.

Table 1. Case-series of horses with wound or history of surgical procedures related with tetanus. Botucatu, SP, Brazil (1990-2015)

Age group	Horse	Age	Sex	Breed	Month/year of occurrence (season)	Wound/predispose condition (days before onset clinical signs)	First signs until hospitalization	Length of hospitalization	Outcome
	1	8 days	M	Mang	Nov/1990 (Spring)	Umbilical infection	1 day	3 days	Died
	2	9 days	M	App	Oct/1995 (Spring)	Umbilical infection	2 days	1 day	Died
	3	13 days	M	MB	Nov/1992 (Spring)	Umbilical infection	1 day	2 days	Died
	4	15 days	M	Mang	Sep/2010 (Spring)	Umbilical infection	3 days	3 days	Died
0-2 years	5	30 days	M	MB	Feb/2000 (Sum)	Umbilical infection	2 days	1 day	Died
	6	5 months	F	Camp	May/2014 (Aut)	Wound in hind limb	5 days	9 days	Survived
	7	9 months	F	MB	Aut/2010 (Sum)	Wound in hind limb	1 day	3 days	Died
	8	2 years	M	MB	Jul/2011 (Wint)	Wound in hind limb	6 days	1 day	Died
	9	2 years	M	MB	Sep/2012 (Spring)	Wound in front limb	2 days	7 days	Survived
	10	2 years	M	QH	Nov/1994 (Spring)	Wound in hind limb	1 day	3 days	Died
	11	3 years	F	Mang	Nov/1990 (Spring)	Wound in hind limb	1 day	8 days	Died
	12	3 years	M	Mang	April/1991 (Aut)	Castration (25 d)	2 days	2 days	Died
	13	3 years	M	QH	May/1991 (Aut)	Contaminated injection	1 day	2 days	Died
	14	3 years	F	App	June/2000 (Aut)	Wound in the legs	10 days	10 days	Survived
3-4 years	15	3 years	M	MB	July/2000 (Wint)	Castration (20 d)	2 days	4 days	Survived
	16	3 years	M	QH	June/2011 (Wint)	Castration (15 d)	2 days	5 days	Survived
	17	3 years	M	NI	Sep/2015 (Spring)	Wound in face (30 d)	5 days	5 days	Died
	18	3 years	M	MB	Nov/2015 (Spring)	Wound in hind limb	15 days	15 days	Survived
	19	4 years	F	QH	Aug/2001 (Wint)	Wound in front limb (20 d)	20 days	1 day	Died
	20	4 years	M	NI	Feb/2003 (Sum)	Wound in hind limb	5 days	18 days	Survived
	21	4 years	F	Mang	Aug/2003 (Wint)	Wound in front limb	15 days	24 days	Survived
	22	5 years	F	MB	Dec/1993 (Spring)	Wound in hind limb	1 day	1 day	Died
	23	5 years	M	Mang	Feb/2003 (Sum)	Wound in hind limb	3 days	12 days	Survived
5-6 years	24	5 years	F	Mang	July/2006 (Wint)	Wound in face	3 days	1 day	Died
	25	5 years	M	MB	Feb/2012 (Sum)	Wound in inguinal region	2 days	1 day	Died
	26	6 years	M	And	Oct/1994 (Spring)	Wound in front limb	7 days	7 days	Survived
	27	6 years	F	MB	June/2011 (Wint)	Wound in face (1 d)	1 day	2 days	Died
	28	7 years	M	Camp	April/1990 (Aut)	Shoeing (30 d)	1 day	1 day	Died
	29	7 years	F	MB	May/1998 (Aut)	Wound in face (20 d)	4 days	1 day	Died
7-8 years	30	7 years	F	MB	May/2014 (Aut)	Wound in hind limb	8 days	2 days	Died
	31	8 years	F	Cri	Nov/1993 (Spring)	Wound in hind limb	3 days	4 days	Survived
	32	8 years	F	NI	March/2002 (Aut)	Wound in hind limb (15 d)	1 day	4 days	Died
	33	8 years	F	MB	Aut/2002 (Spring)	Wound in hind limb	7 days	14 days	Survived
9-10 years	34	10 years	F	MB	Feb/1993 (Sum)	Wound in dorsal region	2 days	2 days	Died
	35	10 years	F	MB	July/1998 (Wint)	Wound in front limb (15 d)	1 day	5 days	Died
	36	11 years	M	QH	Feb/2006 (Sum)	Wound in hind limb	1 day	6 days	Died
	37	11 years	M	Mang	Sep/2009 (Spring)	Wound in hind limb	8 days	2 days	Died
>10 years	38	12 years	M	MB	Aug/2008 (Wint)	Woun in front limb	15 days	6 days	Died
	39	14 years	M	Mang	Dec/2013 (Sum)	Wound in hind limb	2 days	7 days	Survived
	40	16 years	F	MB	April/2004 (Aut)	Wound in hind limb	4 days	1 day	Died

d = day(s), M = male, F = female, And = Andalusian, App = Appaloosa, Camp = Campolina, Cri = Criollo, MB = mixed breed, Mang = Mangalarga, QH = Quarter horse, Aut = Autumn, Sum = Summer; Wint = Winter, Feb = February, Aug = August, Sep = September, Oct = October, Nov = November, Dec = December, NI = no information/missing data.

Table 2. Case-series of horses without wound or history of surgical procedures related with tetanus. Botucatu, SP, Brazil (1990-2015)

Age group	Horse	Age	Sex	Breed	Month / year of occurrence (season)	First signs until hospitalization	Length of hospitalization	Outcome
	1	1 year	M	QH	July/2014 (Winter)	1 day	1 day	Died
	2	1 year	M	QH	Aug/2014 (Winter)	2 days	2 days	Died
0-2 years	3	1 year	F	Mang	March/2015 (Aut)	1 day	2 days	Died

M = male, F = female, Arab = Arabian, Camp = Campolina, MB = mixed breed, Mang = Mangalarga, QH = Quarter horse, Aut = Autumn, Sum = Summer, Jan = January, Feb = February; Aug = August, Sep = September, Nov = November, Dec = December, NI = no information/missing data.

Table 2. Continued...

Age group	Horse	Age	Sex	Breed	Month / year of occurrence (season)	First signs until hospitalization	Length of hospitalization	Outcome
	4	2 years	M	QH	July/1992 (Winter)	1 day	14 days	Survived
	5	2 years	M	MB	April/1995 (Aut)	5 days	1 day	Died
	6	2 years	F	MB	Aut/2000 (Spring)	1 day	10 days	Survived
	7	3 years	M	MB	July/1991 (Winter)	5 days	3 days	Died
	8	3 years	M	MB	Feb/2015 (Sum)	2 days	2 days	Died
3-4 years	9	4 years	M	MB	July/1990 (Winter)	1 day	4 days	Died
	10	4 years	F	MB	Jan/1992 (Sum)	1 day	4 days	Died
	11	4 years	M	Arab	Sep/1996 (Winter)	3 days	10 days	Survived
	12	5 years	M	MB	Sep/2002 (Winter)	3 days	5 days	Died
	13	5 years	M	Mang	Jan/2008 (Sum)	3 days	1 day	Died
5-6 years	14	5 years	F	MB	Aug/2011 (Winter)	1 day	1 day	Died
	15	6 years	F	MB	Nov/1991 (Spring)	1 day	4 days	Died
	16	6 years	F	MB	Sep/2000 (Winter)	1 day	1 day	Died
	17	6 years	F	MB	July/2002 (Winter)	1 day	1 day	Died
	18	7 years	M	QH	Nov/1997 (Spring)	1 day	3 days	Died
7-8 years	19	8 years	M	MB	Sep/1990 (Winter)	10 days	22 days	Survived
	20	8 years	M	MB	May/1998 (Aut)	4 days	3 days	Died
	21	8 days	F	MB	May/2002 (Aut)	3 days	1 day	Died
	22	9 years	M	MB	Sep/1992 (Winter)	1 day	1 day	Died
	23	9 years	M	MB	June/2000 (Aut)	4 days	7 days	Survived
9-10 years	24	9 years	F	MB	May/2001 (Aut)	1 day	1 day	Died
	25	9 years	M	Camp	July/2010 (Winter)	2 days	15 days	Survived
	26	10 years	F	App	Aug/1995 (Winter)	1 day	1 day	Died
	27	11 years	M	Mang	Sep/2009 (Winter)	8 days	3 days	Died
>10 years	28	12 years	F	NI	Aug/2014 (Winter)	2 days	3 days	Died
	29	18 years	M	MB	Dec/2013 (Sum)	2 days	2 days	Died
	30	18 years	F	MB	Nov/2015 (Spring)	5 days	3 days	Died

M = male, F = female, Arab = Arabian, Camp = Campolina, MB = mixed breed, Mang = Mangalarga, QH = Quarter horse, Aut = Autumn, Sum = Summer, Jan = January, Feb = February; Aug = August, Sep = September, Nov = November, Dec = December, NI = no information/missing data.

Table 3. Relation between selected epidemiological data and clinical outcome rate in 70 cases of tetanus in horses. Botucatu, SP, Brazil (1990-2015)

Epidemiological data	Survivors N (%)	Fatalities N (%)	*p* value*
Breed			
Mixed bread	8 (20.0%)	32 (80.0%)	0.15
Mangalarga	3 (25.0%)	9 (75.0%)	
Quarter horse	2 (22.2%)	7 (77.8%)	
Appaloosa	1 (33.3%)	2 (66.7%)	
Campolina	2 (66.7%)	1 (33.3%)	
Other	3 (100.0%)	0 (--)	
Age			
0-2 years	4 (25.0%)	12 (75.0%)	0.59
3-4 years	7 (43.8%)	9 (56.2%)	
5-6 years	2 (16.7%)	10 (83.3%)	
7-8 years	3 (30.0%)	7 (70.0%)	
9-10 years	2 (28.6%)	5 (71.4%)	
>10 years	1 (11.2%)	8 (88.8%)	
Gender			
Female	6 (20.7%)	23 (79.3%)	0.31
Male	13 (31.7%)	28 (68.3%)	

N = number of horses, * Level of statistical significance was set at 0.05, ** Significant association.

Table 3. Continued...

Epidemiological data	Survivors N (%)	Fatalities N (%)	*p* value*
Wound or history of surgical procedures			
	13 (32.5)	27 (67.5%)	0.24
Season			
Spring	6 (33.3%)	12 (66.7%)	
Summer	3 (25.0%)	9 (75.0%)	0.86
Autumn	3 (20.0%)	12 (80.0%)	
Winter	7 (28.0%)	18 (72.0%)	
Incubation period			
≤ 15 days	1 (25.0%)	3 (75.0%)	0.67
> 15 days	1 (16.7%)	5 (83.3%)	
Onset clinical signs and hospitalization			
≤5 days	13 (22.4%)	45 (77.6%)	0.11
>5 days	6 (50.0%)	6 (50.0%)	
Lenght of hospitalization			
<7 days	1 (2.6%)	38 (97.4%)	0.001**
≥7 days	16 (94.1%)	1 (5.9%)	

N = number of horses, * Level of statistical significance was set at 0.05, ** Significant association.

Table 4. Main clinical signs observed at first clinical examination of 70 cases of tetanus in horses. Botucatu, SP, Brazil (1990-2015)

	Absolute frequency	Relative frequency (%)
Hyperesthesia	70	100.0
Limb spasticity	70	100.0
Cervical stiffness	70	100.0
Tetanic spasms (after touch or sound)	67	96.0
Restriction of jaw movements	64	91.4
Sweating	55	78.6
Dilatation of nostrils	51	72.8
Anxious expression	50	71.4
Dysphagia/aphagia	49	70.0
Tachycardia/tachypnea	43	61.4
Prolapse of the third eyelid	39	55.7
Elevated tail	33	47.1
Erect ears	20	29.0
Hyperthermia	18	25.7
Recumbency	11	15.7

DISCUSSION

Routine diagnosis of equine tetanus is based on typical clinical signs, presence of wounds or history of surgical procedures that may predispose to disease (Mackay 2014). Likewise, the diagnosis of tetanus in the horses admitted at veterinary hospital in the current study was based on compatible clinical signs, presence of wounds and history of surgical procedures (Gračner et al. 2015). Due to the inconsistent presence of *C. tetani* and its labile toxins in the foci of the lesions, microbiological exams are generally not assessed (Green et al. 1994). Furthermore, no definitive ante-mortem tests or pathognomonic post-mortem lesions are reliable in the routine diagnosis of tetanus in horses (Kay & Knottenbelt 2007).

The frequency of 1.21% of disease in this case series fit with other similar retrospective studies, which reported about 1% or less of the caseload in Veterinary Hospitals elsewhere (Kay & Knottenbelt 2007, Green et al. 1994). Nevertheless, another similar study in Brazil, 5% of the cases were reported in equines used in cart pushing, a fact attributed to poor social and economic conditions of owners and improper/absence vaccination of animals (Reichmann et al. 2008). In addition, data assessed from horses in the current study was provided by a convenience sampling, which may be considered a main limitation because is not representative of Brazil.

Mortality rates of about 50% were recorded in most retrospective studies involving tetanus in horses (Reichmann et al. 2008, Van Galen et al. 2008). High mortality caused by tetanus in equines have been attributed to the dose of clostridia inoculation and toxin production, poor body conditions and immune status of animals, lack or improper vaccination (Green et al. 1994), therapeutic procedures and veterinary assistance only long time after the onset of clinical signs, high susceptibility of horses to the tetanus toxin (Kay & Knottenbelt 2007), inappropriate horse care (Gračner et al. 2015), and nursing care limitations in affected horses (Van Galen et al. 2008). In this scenario, the high mortality rate (72.9%) observed in the present case series was expected given mainly the absence of vaccination status in all enrolled horses. Another retrospective study, with 20 cases of tetanus-affected horses, showed that none of non-vaccinated animals survived (Green et al. 1994). In fact, an improper vaccination program or absence of active immunization against equine tetanus has been strongly related to poor prognosis and, consequently, higher rates of mortality (Reichmann et al. 2008).

The clinical outcomes in horses of our case series was not influenced by some epidemiological factors analyzed, which is

consistent with similar previous retrospective studies (Kay & Knottenbelt 2007, Reichmann et al. 2008, Gračner et al. 2015). Nonetheless, greater vulnerability (Green et al. 1994) and poor prognosis (Van Galen et al. 2008) were cited for young horses affected by tetanus. Similarly, it is important to note in the current study that 90% of foals until 1-year-old died, especially neonatal animals (until 30 days of age), probably because unnvacinatted young horses are more vulnerable to infectious diseases such as tetanus due improper immunity response (Wilson et al. 2001).

No clear association was found between the outcome and the occurrence of tetanus in the different seasons. Thus, a probably suitable situation for the development of the tetanus in horses is provided by the climate and soil conditions found in the studied region, particularly warm temperatures and intensively farmed areas, which contribute with the persistence of spores in the environment and their viability in the contamination of wounds, favouring conditions to occurrence of disease over the year in tropical countries (Mackay 2014).

Spores of *C. tetani* are a highly resistant life form of the pathogen and are formed in unfavorable situations, particularly when aerobic conditions are found (Quinn at al. 2011). Spores can persist in soil or the environment for many years and traumatic inoculation of spores through puncture wounds is the most common port of entry (Mackay 2014). Lesions are frequently contaminated by manure, soil, as well as surgical equipment or horse management tools. In addition, infection of the umbilical cord, improper injections or castration procedures are possible routes of infection by spores of *C. tetani* in livestock species (Kay & Knottenbelt 2007). However, apparent lesions or other predisposing conditions are frequently not well-determined or are unnoticed in some equine tetanus cases (Reichmann et al. 2008).

In this study, forty horses presented history of surgical procedures related with tetanus (umbilical infections, castration, recent injection, shoeing) or showed visible injuries prior to the onset of the clinical signs, particularly involving hind and front limbs wounds; a finding that is in agreement with other retrospective studies of equine tetanus that reported skin injuries in the extremities of the limbs as the most important lesion related to the disease (Green et al. 1994, Reichmann et al. 2008). No association was observed between the outcome and the presence of wounds or that was noticed previous surgical procedures. However, all nine horses with history of umbilical infections and wounds in the face evolved to obit, and these data were considered a circumstantial evidence of poor prognosis. Wounds and predisposing conditions showed no influence in the prognosis of clinical outcomes in a retrospective study with 76 horses in Brazil (Reichmann et al. 2008), as opposed to a study in Croatia that described the presence of obvious wound as having a statistical effect on mortality (Gračner et al. 2015). Nonetheless, this epidemiological parameter should be critically analyzed because, in many instances, information on wounds or history of surgical procedures is not precise (Kay & Knottenbelt 2007), and there are difficulties in the identification of minor wounds (Stämpfli 2016), particularly on the soles of feet, a condition that favors the establishment of anaerobic conditions and production of tetanus toxins (Reichmann et al. 2008).

Thirty horses admitted at the Veterinary Hospital in the current study showed no visible wounds or previous surgical procedures. These data are in agreement with most retrospective studies involving equine tetanus, in which lesions or history of surgical procedures that may predispose to disease were not noticed or identified in about 30% or more animals (Kay & Knottenbelt 2007, Reichmann et al. 2008, Van Galen et al. 2008, Gračner et al. 2015). The absence of visible wounds at admission, so called "idiopathic tetanus", occur occasionally in domestic herbivores associated with consumption of fibrous or rough feed, possibly leading toxin production in wounds located in the mouth or intestinal tract (Constable et al. 2016).

The incubation period of equine tetanus usually lasts between 7 to 21 days, although some animals may present clinical signs as late as months after the initial infection (Mackay 2014). Fatal course of illness usually occurs 5 to 10 days after the onset of clinical manifestations. In ten horses of the current study, mean time from infection by *C. tetani* to development of typical clinical signs was 19.1 days. However, in a series of 18 cases of equine tetanus, mean time from the history of the wound to the onset of clinical signs was 9 days (Green et al. 1994). Another retrospective study reported an incubation period of 15 days in 16 cases of equine tetanus (Van Galen et al. 2008). Usually, a long incubation period is associated with a mild syndrome and good prognosis. In turn, a short incubation period and faster progress of clinical signs is usually related to poor prognosis (Constable et al. 2016), probably because influence of infective dose or distance from port to entry to central nervous system (CNS) (Shumacker et al. 1939). In this context, the short distance between face and CNS could explain, in part, fatal evolution of cases in the current study with presence of wounds in this region of body. Nevertheless, no clear association was found between clinical outcomes and the incubation period among horses studied, although these data should be evaluated with caution, since the assessment of the period between probable infection by *C. tetani* and the onset of first clinical signs was only possible in ten horses.

Retrospective studies involving equine tetanus have reported a great variety of frequency of clinical signs worldwide (Reichmann et al. 2008, Di Filippo et al., 2016), usually without any association with outcomes (Gračner et al. 2015). Limb spasticity, muscle stiffness, hyperesthesia, tetanic spasms, trismus, sweating, difficulty in walking, prolapse of the third eyelid, and tachycardia/tachypnea (Reichmann et al. 2008), erect ears and tail, anxious expression, dilatation of the nostrils (Constable et al. 2016), recumbency, opisthotonos, and saw-horse posture (Gračner et al. 2015) are common clinical signs of equine tetanus. Among our 70 horses, hyperesthesia, limb spasticity, cervical stiffness, tetanic spasms, and restriction of jaw movements were the major clinical signs observed at first clinical examination. The relationship between prolonged recumbency, sweating, and dysphagia/aphagia with the mortality in 51 horses of the current study is noteworthy. These clinical data are consistent with similar retrospective case series of tetanus in horses (Green et al. 1994), and are strongly indicative of poor prognosis (Van Galen et al. 2008). Moreover, the severity of clinical signs was reported as a clear indication of poor prognosis elsewhere (Kay & Knottenbelt 2007), although this clinical parameter was not assessed in our 70 cases.

In this case series, a tendency towards mortality and short time from the first clinical signs (< 5 days) to hospitalization was observed and, consequently, shorter course of the disease was an circumstantial evidence of poor prognosis probably due to more severe clinical signs and/or more rapid progression of disease. This result is in agreement with similar studies in Brazil (Reichmann et al. 2008) and Croatia (Gračner et al. 2015), in which it was reported that the first week of the disease is a critical period for survival in tetanus-affected equines. Differently, other retrospective studies elsewhere reported low prognostic value between the time of onset of clinical signs and initial veterinary assistance (Kay & Knottenbelt 2007, Van Galen et al. 2008).

The major principles of treatment are elimination of the causal agent (antimicrobials), neutralization of tetanus antitoxin, maintenance of hydroelectrolytic balance, cleaning and antiseptic care of wounds, administration of sedative and muscle relaxants, as well as maintenance of the animal in a quiet and comfortable environment (Kay & Knottenbelt 2007, Di Filippo et al., 2016, Constable et al. 2016). In addition, vaccination has been indicated as an additional therapeutic measure for equines affected by tetanus (Reichmann et al. 2008). The treatment adopted was similar in all 70 horses studied and emphasis was given in eliminating the causal agent, neutralizing exotoxins, proper care of wounds, and maintaining the animal in a quiet and comfortable conditons (Green et al. 1994). Exceptions were made to the individual needs for administration of sedatives and muscle relaxants, as well as needs hydroelectrolytic balance (Mackay 2014). No influence was observed between treatment and clinical outcomes in our 70 cases. Indeed, similar retrospective case series failed to establish a relationship between the outcome and some differences among therapeutic protocols of equine tetanus (Kay & Knottenbelt 2007, Reichmann et al. 2008), besides controversial statements about doses and routes of administration that may be more effective in the use of tetanus antitoxin (Kabura et al. 2006, Mackay 2014, Hirano et al. 2016). These results indicate that early diagnosis, use of antimicrobials, nursing procedures, hydration, control of tetanic spasms, cleaning and use of antiseptic solutions in wounds, as well as keeping horses in a quiet and comfortable environment are critical points to successful therapy (Constable et al. 2016).

Here, a longer length of hospitalization of horses affected with tetanus was seen as a parameter of good prognosis, since among the 19 survivors, 16 (84.2%) presented > 7 days of hospitalization. In fact, other similar retrospective studies have reported that 7 days or more of hospitalization for equines have been associated with increase of survival rates (Kay & Knottenbelt 2007, Reichmann et al. 2008). In contrast, fast evolution to recumbency in up to 48 hours has been a predictive parameter of poor prognosis (Green et al. 1994).

Due high mortality rates of tetanus affected-horses (72.9%), even under recommended treatment and nursing care, as well as the absence of vaccination among our 70 cases, there is an obvious need for additional education of horse owners regarding the disease (Ireland et al. 2013), particularly towards the knowledge on active immunization of animals as a crucial tool for prevention (Davis et al. 2015, Di Filippo et al., 2016, Kendall et al. 2016). In this context, in newborn animals from vaccinated mares three-dose series of tetanus toxoid are recommended, beginning at 4 to 6 months of age, with a second dose 4 to 6 weeks later, and a third dose at 11 to 12 months of age (Mackay 2014), as passive immunity of colostrum may interfere with the active immunization of neonates 4 to 6 months of age (Green et al. 1994, Wilson et al. 2001,). Conversely, the vaccination of foals born from non-vaccinated mares should begin at 1 to 4 months of age, and a 3-dose protocol at 4-week intervals is recommended. Traditionally, non-vaccinated horses are immunized with 2 doses of toxoid 3 to 6 weeks apart. Pregnant mares are revaccinated 4 to 6 weeks before foaling, to ensure suitable colostrum passive immunity to foals (Mackay 2014). Although immunity to tetanus lasts about 5 years, annual revaccinations are a common practice for equines (Constable et al. 2016). Revaccinations are recommended on the occurrence of wounds or before surgical procedures. In addition to a proper vaccination program and education of horse owners in the studied region with regards to the main aspects of tetanus (Reichmann et al. 2008, Gračner et al. 2015) the following strategies are suggested to control and prevention of the disease: suitable availability of food over the whole year, diagnosis of disease and prompt therapy as early as possible, proper cleaning and antisepsis of wounds and umbilical region in neonates, and use of the best aseptic techniques before injections, castration or other surgical procedures.

CONCLUSIONS

We observed that presence of umbilical infections, wounds on the face, prolonged recumbency, sweating, dysphagia/aphagia, and <5 days between onset of first clinical signs until prompt veterinary assistance displayed a circumstantial evidence of poor prognosis; whereas a significant association (p=0.001) between survival and >7 days of length of hospitalization was predictive of good prognosis.

The high mortality rate, even in horses with specific treatment, highlight the need for early diagnosis, prompt veterinary assistance, and establishment of prophylatic measures against tetanus in horses.

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