

Diagnosis of Aspergillosis in Horses

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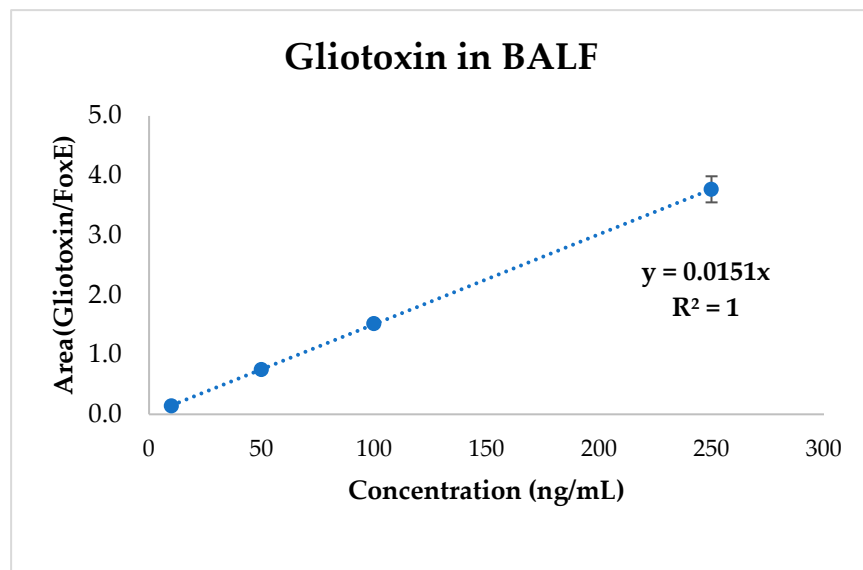
Supplementary Table S1: Review of previously reported cases of invasive pulmonary aspergillosis in horses.

Country	No. of cases in the study	No. of cases/Breed	Age	Sex	Causative agent	Underlying condition	Diagnosis	Outcome	Reference
Canada	1	1 SB	20 years	Mare	<i>Aspergillus fumigatus</i>	Hepatopathy susp.	PM	death	Long et Mitchel 1971
USA (N.Y.)	1	1 SB	2 years	Filly	<i>Aspergillus</i> spp.	Myelomonocytic leukemia	PM	euthanasia	Blue et al. 1987
USA (Florida)	1	1 QH	7 months	Colt	<i>Aspergillus fumigatus</i>	Diarrhea, laminitis, bacterial uveitis	PM	death	Green et al. 1987
USA (Michigan, N.Y.), Australia	19	(5 TB, 4 QH, 2 Arabian, 2 Appaloosa, 2 Grade, 1 Saddlebred, 1 Palomino, 1 Standardbred, 1 Welsh)	1 month – 15 years	(Stallions 8, Mares 8, Geldings 3)	<i>Aspergillus</i> spp. (2 of them <i>Aspergillus fumigatus</i>)	(Diarrhea – 14, Pneumonia – 3, Umbilical hernia repair – 1, Laminitis – 3, Septicemia – 1, Obstructive urolithiasis – 1, Myelogenous leukemia – 1, Anemia and ataxia – 1, Corticosteroid treatment – 3)	PM	death/ euthanasia	Slocombe et Slauson 1988
USA (Pennsylvania)	1	1 QH	8 years	Mare	<i>Aspergillus</i> spp.	Enteritis/colitis	PM	death	Hattel et al. 1991
USA (Ohio)	1	1 National Show Horse	4 years	Gelding	<i>Aspergillus</i> spp.	<i>Streptococcus zooepidemicus</i> Infection	IV susp.	euthanasia	Moore et al. 1993
USA (Missouri)	1	1 QH	7 years	Gelding	<i>Aspergillus</i> spp.	Large colon volvulus (surgery), diarrhea	PM	euthanasia	Pace et al. 1994
USA (Michigan)	1	1 TB x QH	11 years	Gelding	<i>Aspergillus</i> spp.	Myelomonocytic leukemia	PM	death	Buechner-Maxwell et al. 1994
Spain	1	1 French Saddlebred	30 years	Mare	<i>Aspergillus fumigatus</i>	Cushing syndrome	PM	euthanasia	Carrasco et al. 1996
USA (Michigan)	1	1 QH	6 years	Mare	<i>Aspergillus</i> spp.	Enterocolitis	IV susp.	death	Rosenstein et Mullaney 1996
Spain	1	1 TB	2 years		<i>Aspergillus niger</i>	<i>Rhizopus solonifer</i> pneumonia	PM	death	Carrasco et al. 1997
USA (Pennsylvania)	29	(10 TB, 11 SB, 2 QH, 2 Western, 1 Appaloosa, 1 Saddlebred, 1 Arabian, 1 Pony)	5.0 ± 4.4 years	(Mare 15, Stallion 7, Gelding 7)	<i>Aspergillus</i> spp.	[Primary GIT disease – 20, Secondary GIT disease – 5 (diarrhea 12, colic 4, weight loss 2, others 1 each: laryngoplasty, renal failure, sinus surgery, pleuropneumonia, myositis, DIC, toxemia, hepatic failure, ataxia, debilitation)]	IV – 1 IV susp. – 1 PM - 27	euthanasia 17 death 12	Sweeney et Habecker 1999
USA (Missouri)	2	(1 QH, 1 Arabian)	2 - 14 years	(Gelding, Stallion)	<i>Aspergillus</i> spp.	[Ataxia (EPM) Strangulation of small intestine (surgery)]	PM PM	death death	Johnson et al. 1999
Iran	1	N/A	7 years	N/A	<i>Aspergillus fumigatus</i>	Purulent pneumonia	PM	death	Tafti et al. 2001
France	1	1 Pony	6 years	Mare	<i>Aspergillus fumigatus</i>	Colitis, mucormycosis	PM	death	Thirion-Delalande et al. 2005
USA (California)	1	1 TB	14 days	Colt	<i>Aspergillus fumigatus</i>	Diarrhoea (enterocolitis)	IV	healing after surgical resection of lesion and voriconazole administration	Hilton et al. 2009
Brazil	2	(1 Brasileiro-de-Hipismo, 1 PH)	1.5 - 3 years	(Filly 1, Colt 1)	<i>Aspergillus fumigatus</i> both horses	Both horses: anaemia, apathy, fever, neurological signs, poor body condition	PM PM	death death	Headley et al. 2014

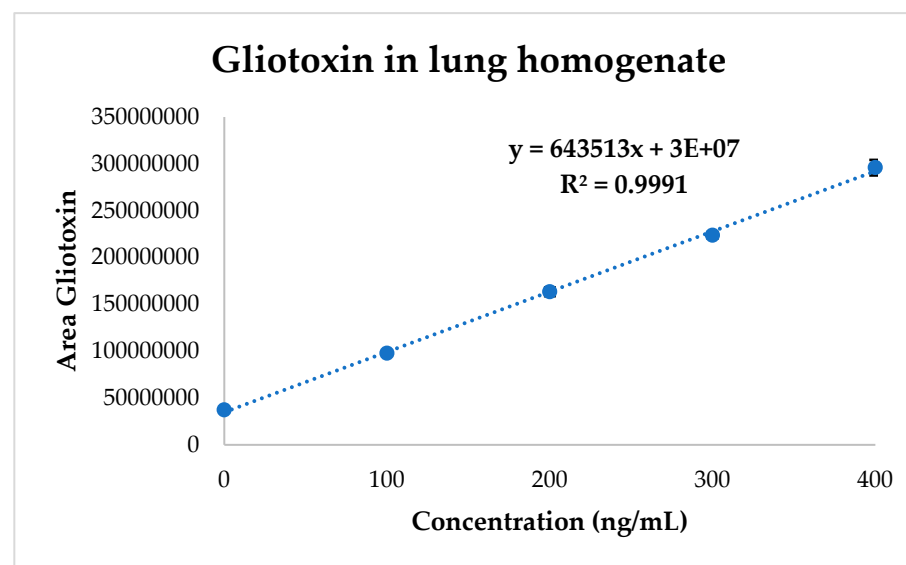
SB, Standardbred; QH, Quarter Horse; TB, Thoroughbred; PH, Paint Horse; PM, postmortem; IV, *in vitro*; susp., suspicious; EPM, equine protozoal myelitis; N/A, not available

Supplementary Table S2. Calibration curves for gliotoxin and ferricrocin. Calibration linearity and regression coefficients (R^2) are shown in the insets. For quantitation of gliotoxin in bronchoalveolar lavage fluid, the calibration curve (blank, 1, 5, 10, 50, 100, 250 ng/mL) was constructed from $[M+H]^+$ ion signals (a). The instrumental LOD and LOQ were 2.1 ng/mL and 6.5 ng/mL respectively. The content of gliotoxin in lung tissue homogenate was determined by use of prepared standards (0, 100, 200, 300, 400 ng/mL) (b). Ferricrocin calibration (blank, 1, 5, 10, 50, 100, 250 ng/mL) was plotted using $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ ion signals with the instrumental LOD and LOQ, 0.6 ng/mL and 1.9 ng/mL respectively (c).

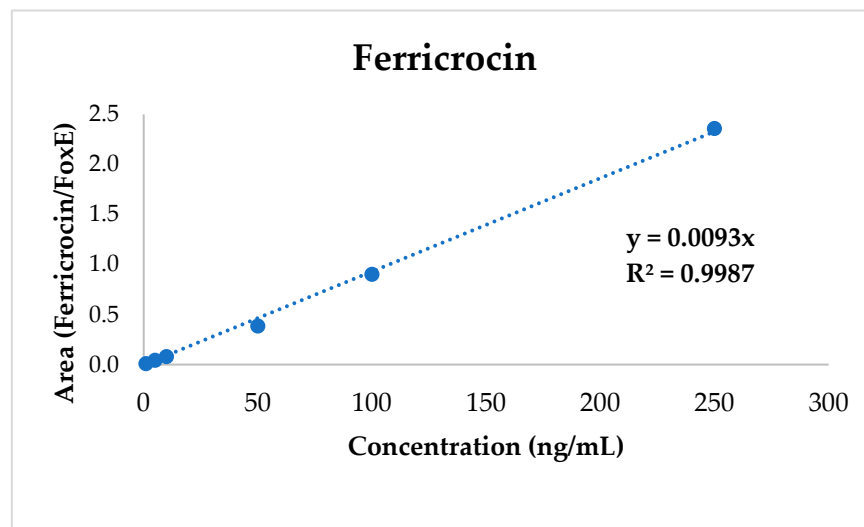
a



b



c



Supplementary Table S3. Characteristics of horses with underlying pulmonary disease, healthy controls and horse with IPA. Results of *Aspergillus* spp. detection in tracheal wash and BALF. IPA, invasive pulmonary aspergillosis; EHV5, Equine Herpes Virus 5; BALF, Bronchoalveolar Lavage Fluid. Difference between the severe asthma, moderate asthma, IPA and the healthy control group with regard to the occurrence of *Aspergillus* spp. in BALF and tracheal wash was statistically significant (P<0.05).

	Total enrolled (n=18)	Severe Asthma (n= 8)	Moderate Asthma (n = 4)	IPA, EHV 5, Colitis, Death (n=1)	Healthy (n=5)
Age - mean (range)	12 (3-22)	11.9 (9-20)	11.5 (8-14)	3 (3)	10.2 (3-22)
Stallions (%)	2 (11)	1 (13)	0 (0)	1 (100)	0 (0)
Mares (%)	7 (39)	2 (25)	2 (50)	0 (0)	3 (60)
Geldings (%)	9 (50)	5 (63)	2 (50)	0 (0)	2 (40)
Tracheal wash culture:					
<i>Aspergillus fumigatus</i>		4	1	Not sampled	1
<i>Aspergillus montevideensis</i>		2	1	None	None
<i>Aspergillus flavus</i>		1	None	None	None
<i>Aspergillus niger</i>		4	1	None	None
<i>Aspergillus nidulans</i>		1	1	None	None
<i>Aspergillus chevalieri</i>		1	None	None	None
BALF culture:					
<i>Aspergillus fumigatus</i>		1	1	1	None
<i>Aspergillus montevideensis</i>		3	1	None	None
<i>Aspergillus flavus</i>		1	None	None	None
<i>Aspergillus niger</i>		1	None	None	None
<i>Aspergillus nidulans</i>		None	None	None	None
<i>Aspergillus chevalieri</i>		1	None	None	None
Tracheal wash + BALF identical finding:					
<i>Aspergillus fumigatus</i>		1	1	-	None
<i>Aspergillus montevideensis</i>		2	1	None	None
<i>Aspergillus flavus</i>		None	None	None	None
<i>Aspergillus niger</i>		None	None	None	None
<i>Aspergillus nidulans</i>		None	None	None	None
<i>Aspergillus chevalieri</i>		1	None	None	None