Effects of lambda-carrageenan on equid herpesvirus 3 in vitro

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Batch date on straw (dd/mm/yy)	qPCR assay result		
	Positive (Ct ≤ 40)	Negative (Ct > 40)	Total
18/09/2006	1	7	8
19/09/2006	6	4	10
29/09/2006	8	0	8
06/10/2008	1	0	1
16/08/2010	21	1	22

Semen straws (n)	Sensitivity (%)	Lower CI (%)
1	0.75	0.43
3	0.98	0.81
5	0.99	0.94
7	0.99	0.98
10	0.99	0.99

undefined [1]. This study tested the sensitivity of a RT-qPCR assay for detecting *T. equigenitalis* DNA in frozen-thawed semen collected from a Lipizzaner carrier-stallion identified during epidemiological investigation of the first South African CEM outbreak in 2011 [2]. This stallion was recently linked to his cryopreserved semen collected between 2006-2010 prior to outbreak recognition. Forty-nine 0.5 ml PVC straws from five ejaculates (batches) processed using a cryopreservative extender with added antimicrobials were recovered. All straws were thawed and aliquots from each were transferred for both bacterial isolation and qPCR assay using a modification of an established method [3]. The assay results summarised in Table 1 below showed 37/49 (75.5%) straws were PCR-positive (Ct ≤40, range: 30.9-40). Bacterial isolation from all straws was negative.

Sensitivity of the qPCR assay for detecting *T. equigenitalis* DNA in individual frozen-thawed straws (n=49) from five ejaculates was estimated as $1-(1\text{-}p)^n$ where p = overall proportion of individual qPCR positive straws and n = number of straws tested (Table 2). 95% Confidence Intervals were adjusted for the clustering of observations in the five ejaculates.

The qPCR assay effectively identified *T. equigenitalis* DNA contaminating multiple straws of different ejaculates processed over four years. Extensive variation in Ct values within and between batches was possibly due to effects of semen collection and processing including addition of antibiotic-containing extender. In conclusion, this data suggested that ≥ three straws per batch should be evaluated for 95% confidence of detecting *T. equigenitalis* in cryopreserved semen from a potential carrier stallion but the lower range of the 95% CI suggests a larger study is necessary to improve precision of this estimate. Infectivity of the bacterium after cryopreservation warrants further investigation.

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Re-emerging Equine Arteritis Virus (EAV) variants

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Equine viral arteritis (EVA), caused by the Equine Arteritis Virus (EAV), is a disease of stallions notifiable in the UK and to the OIE, but not in most other countries. However, with no active surveillance in most countries (including the EU) and the clinical presentation mostly asymptomatic, EAV is often first noticed when causing issues for horses supposed to travel or to enter breeding schemes. Although there is only one serotype of EAV, genetic variation exists between field strains of the virus. A segregation of strains into European and North American lineages, wherein the European lineage can be further divided into two clusters has been attempted. Unsurprisingly, however, some European lineage viruses have been documented in North America and vice versa, demonstrating the limits of this nomenclature as well as highlighting the transport of viruses via movement of horses and semen. Over the last years we discovered a couple of EVA cases through the analysis of horses that had been bought from continental Europe or were to be sold in the UK two of which shall be presented here. In September 2012 we analysed a semen sample from a stallion imported from Spain, which tested positive for EAV. Initial analysis of the ORF5 PCR product revealed the isolate to be an outlier to previous phylogentic grouping, together with KY63 and H21. This demonstrates the re-emergence of a genotype across decades and around the globe: KY63 is a field strain isolated in Kentucky in 1963; this isolate has always clustered separately from other US isolates of the same time period suggesting a different origin. Strain H21 appears to have been isolated in the early 2000's in Hungary. Even considering a potential lack of sampling the case demonstrates that established strains do not become extinct and through global trade may re-appear unexpectedly, here in Spain (UK) rather than Eastern Europe or the USA. The role of semen in the transmission has been demonstrated in several outbreaks recently, both in North and South America, as well as in Europe. Previously, we analyzed another case of an imported stallion from continental Europe. To our surprise this virus was very closely related to the virus that has been linked to the Argentinian outbreak in the same year, but as further analysis demonstrated was not the only variant of EAV circulating at the time in Europe. Therefore, highly similar and dissimilar variants of EAV are circulating in parallel, using largely unexplored reservoirs for their maintenance. Using all available data on full genome and ORF5 sequences a dynamic extended systematics is proposed for EAV.

Posters
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Effects of lambda-carrageenan on equid herpesvirus 3 *in vitro*

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Equine Coital Exanthema (ECE) is an infectious, venereally transmitted mucocutaneous disease of mares and stallions caused by Equid herpesvirus 3 (EHV3). Episodes of reactivation and re-excretion in latently infected horses serve as a source of virus, and thus the infection is endemic in horses worldwide. Prevention for stallions is based on segregating affected mares from reproduction. However, as the virus is reactivated from latently infected animals and generally re-excreted subclinically, and this is not predictable, the prevention approach previously described does not eliminate the risk of contagion. Lambda-carrageenans (λ-carrageenans), which are sulfated polysaccharides synthesized in great quantities by certain red seaweed *Gigartina skottsbergii* (Gigartinaceae, Rhodophyta), have demonstrated antiviral activity *in vitro* against Human herpesvirus 1(HHV1) and 2 (HHV2) and some herpesviruses of veterinary interest such as Bovine herpesvirus 1 (BoHV1), Suid herpesvirus 1 (SuHV1) and Feline herpesvirus 1 (FeHV1). The antiviral activity of λ-carrageenan is likely due to its ability to bind to the viral envelope glycoproteins and prevent virus binding to cell surface receptors. The purpose of this study was to evaluate the activity of λ-carrageenans on EHV-3 *in vitro*. Confluent monolayers of EDerm cells were infected with 590 plaque forming units (PFU) of EHV-3 per well, simultaneously, with different concentrations of λ-carrageenans in a total volume of 500 μl. After 1 h of incubation at 37°C, inoculum was discarded and minimum essential medium supplemented with carboxymethyl cellulose 0.75% was added. Cells infected with the virus without λ-carrageenans were used as a control. After 72 h of incubation, cells were fixed and stained with 0.1% formalin-buffered crystal violet solution. Inhibitory concentrations 50 (IC50%) and 100 (IC100%), defined as the concentrations of the compound that reduce plaque number by 50% and 100% respectively, were determined. The IC50% of this compound was 6.22 μg/ml but the IC100% could not be reached, even at a concentration as high as 1g/ml. The IC50% (6.22 μg/ml) found in this study for λ-carrageenans against EHV3 is higher than that found for HHV1 (1.6 μg/mL), HHV2 (1.5 μg/mL), BoHV1 (0.52 μg/ml) and FeHV1 (5 μg/ml), but smaller than that found for SuHV1 (10.42 μg/ml). According to our studies, λ-carragenans do not inhibit replication (100% inhibition) of EHV3 *in vitro*; thus, this compound used as a single component appears to be not eligible for the use in the prevention and treatment of ECE.

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Addition of an intramammary antimicrobial formulation markedly decreased the intervals to elimination of *Taylorella equigenitalis* in a carrier mare and gelding

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Based on the successful treatment of 27 *T. equigentitalis*-carrier horses using a standard topical antimicrobial protocol during a recent CEM outbreak [1] we investigated the addition of a proprietary intramammary antimicrobial formulation for treating two subsequently-identified carriers; a mare and gelding. This combination aimed to reduce the interval to treatment endpoint supported by its reported residual effect and design facilitating application in the bacterium's predilection sites. The gelding was sedated to allow extrusion of the penis before cleaning with a surfactant (Ducosol®) and scrubbing with 4% chlorhexidine gluconate (Dismed Bioscrub®). The urethral fossa was thoroughly cleaned using chlorhexidine-soaked cotton-tipped bacteriology swabs. A dry cow intramammary ointment (Curaclox®) containing cloxacillin benzathine (600mg) and ampicillin (300mg) was instilled into the urethral fossa and sinus before covering the penis with 0.2% nitrofurazone (Furex®) ointment. In the mare, the clitoral sinus and fossa were similarly cleaned with Dismed Bioscrub® before thorough cleansing with chlorhexidine-soaked cotton-tipped swabs (clitoral fossa) and paediatric swabs (clitoral sinus). Thereafter Curaclox® was instilled into the clitoral sinus and Furex® applied to the clitoral fossa. Prior to once-daily treatment, genital swabs of all predilection sites were obtained for detecting *T. equigenitalis* DNA using a modification of a previously-described qPCR assay [2]. Treatment endpoint was defined as three consecutive days of Ct > 40 on assay and confirmed by bacteriology after a 21 day-interval. The table below shows the interval to elimination of *T. equigenitalis* determined by qPCR assay of genital swabs obtained from 27 previously-treated horses (standard protocol) and the mare and gelding treated with the addition of Curaclox® (combination treatment).

Treated horses	Interval to elimination of *T. equigenitalis* (days)
A. Standard topical protocol	
Lipizzaner (stallions: n=23; gelding: n=1)	median: 11; range: 9-52
Warmblood (stallion: n=1)	10
Andalusian (stallion: n=1)	9
Thoroughbred (mare: n=1)	14
B. Standard topical protocol plus Curaclox® instillation	
Warmblood (stallion: n=1)	2
Warmblood (mare: n=1)	2

In conclusion these preliminary data showed a marked reduction in the interval to elimination of *T. equigenitalis* in two horses with the combination treatment. The potential benefits associated with this promising outcome include economical and animal welfare considerations and support its further investigation.

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