Figure 1. Twenty-five years of NPA cases (1133) in Kentucky.

tissue in 2002. In 2003, three species of *Amycolatopsis* and in 2009 two species of *Streptomyces* were identified in association with NPA cases. *Amycolatopsis spp.* (48.7%) and *Crossiella equi* (28.9%) were the most prominent nocardioforms identified in a recent abortion outbreak. This infection leads to late abortions, stillbirths and premature foaling. Premature foals sometimes die shortly after birth. The mode of transmission of this syndrome is not known. To date, nocardioform *Actinomycetes* have only been isolated from placental tissue. Most reported cases are from central Kentucky (Figure 1) but cases have also been diagnosed in Florida, Italy, and South Africa. In the 2010-2011 equine reproductive season, our Lexington laboratory diagnosed 118 cases of NPA by culture and PCR. Due to the high incidence of NPA that season, a farm-level study was conducted to identify possible risk factors for NPA. A total of 148 horse farms were included in a survey (98 affected, 50 farms unaffected). In total, 8075 mares were at risk on all farms with 429 mares diagnosed with NPA. On average, four mares were affected per farm. Data were analysed using chi-squared, Wilcoxon rank-sum tests, and logistic regression analysis using S-PLUS. Farm acreage, mare numbers, and higher density on affected farms were significantly and positively associated with NPA. Longer grazing times during January-March administration of progesterone pre-breeding, HCG post-breeding, and NSAIDs were significantly and negatively associated with NPA. A study at the University of Kentucky attempted to induce NPA in pregnant and non-pregnant mares via intrauterine, oral, and intranasopharyngeal inoculation of *Crosiella equi.* However, these attempts did not result in infections or abortions.

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Contagious Equine Metritis: Efficacy of US post-entry testing protocols for identifying carrier stallions and mares

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Contagious equine metritis (CEM) is a venereally transmissible disease of equids. The etiologic bacterium, *Taylorella equigenitalis*, can cause widespread short-term infertility and very rarely, abortion in mares. A frequent sequel to exposure of stallions and mares to *T. equigenitalis* is establishment of a carrier state that is often long-term in stallions. Aims of this study are twofold: 1) to estimate frequency of the carrier state in stallions and mares by states testing the greatest number of imported horses for CEM; and 2) to establish the basis of determination of persistence of *T. equigenitalis* in individual carrier animals. Test subjects were stallions and mares imported between 1997 and 2014. Mares and stallions underwent post-entry quarantine and testing for CEM in accordance with USDA prescribed protocols. Findings of the study confirmed that there was a continuing risk of reintroduction of CEM into the USA from known CEM-affected countries. Over the 17-year study period, 38 stallions and mares were confirmed carriers of *T. equigenitalis*. Twenty-seven were stallions and 11 were mares. Some 24 of the carrier stallions and all 11 of the carrier mares were identified by the six selected states. There were two instances, both involving stallions, where the post-entry testing protocol failed to detect the carrier state prior to the stallion's release from quarantine. Only eight of the 27 carrier stallions were detected by culturing a single set of swabs. Detection of the carrier state in 18 stallions was only achieved by test breeding. The preponderance (80%) of *T. equigenitalis* strains isolated either from stallions or mares were streptomycin sensitive. The overall positive rate for stallions was 0.98% (24 of 2,457 tested), whereas the corresponding rate for mares was 0.07% (11 out of 15,732). Test breeding as opposed to sole use of culture was a highly reliable but not totally foolproof means of identifying the carrier stallion. A fully validated, less costly, more rapid, and logistically less challenging *in vitro* test is sorely needed for detection of the carrier state, especially in the stallion.

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Sensitivity of qPCR for screening cryopreserved semen from *Taylorella equigenitalis*-carrier stallions

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The risk for disease transmission *via* cryopreserved semen contaminated with *Taylorella equigenitalis* remains largely

Batch date on straw (dd/mm/yy)	qPCR assay result		
	Positive (Ct $\leq$ 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 $\leq$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 $1-(1-p)^n$ 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 $\geq$ 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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