Journal of Equine Veterinary Science 51 (2017) 46–53

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Late-Term Abortion, Stillbirth, and Neonatal Foal Death in Kyrgyzstan: First Isolation of Equine Herpesvirus Type 1 in the Country

Maksat Akhmedzhanov [a,b], Rysbek Nurgaziev [b], Jailobek Orozov [b], Irmgard Moser [c], Nikolaus Osterrieder [a], Armando Mario Damiani [a,d,*]

[a] Institut für Virologie, Zentrum für Infektionsmedizin, Robert von Ostertag-Haus, Freie Universität Berlin, Berlin, Germany
[b] Laboratory of Virology and Biotechnology, Kyrgyz Research Veterinary Institute named after A. Duisheev, Bishkek, Kyrgyzstan
[c] Institut für Molekulare Pathogenese, Friedrich-Loeffler-Institut, Jena, Germany
[d] Instituto de Medicina y Biología Experimental de Cuyo IMBECU, CCT Mendoza, CONICET, Área de Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina

ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form 17 November 2016
Accepted 21 December 2016
Available online 9 January 2017

Keywords:
Abortion
EHV-1
Kyrgyzstan
Mare

ABSTRACT

Late-term abortion, stillbirth, and neonatal foal mortality (commonly referred to as late fetal losses [LFLs]) of unknown etiology are a serious concern for horse farmers and breeders in Kyrgyzstan. Therefore, we investigated major infectious causes of LFL, with a focus on viral agents, for the first time in the horse population in the country. A total of 221 sera and 149 nasal swabs taken from mares in farms with a history of LFL within 1 year of testing as well as tissue samples collected from 17 LFL cases were investigated. Serologic studies revealed that equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4) were prevalent in the population, although positivity rates were low with neutralizing antibody titers of 1:4 to 1:32 (median 1:8) against both pathogens. High antibody titers in the range from 1:32 to 1:512 (median 1:256) against equine arteritis virus (EAV) were detected in mares on a single farm, whereas three mares from different farms tested positive for Dourine. Virus isolation and PCR investigations of nasal swabs did not suggest ongoing active infection with EHV-1, EHV-4, or EAV in the examined mares. Bacteriologic and virological examination of tissue samples taken from LFL cases revealed the presence of *Escherichia coli* and/or *Streptococcus equi* (subsp. *zooepidemicus*) in 35% of neonatal foal death cases, and EHV-1 was isolated from a late-term abortion case representing the first isolation of this important pathogen of horses in the country and, to the best of our knowledge, in Central Asia.

1. Introduction

Kyrgyzstan is a landlocked country in Central Asia, surrounded by Kazakhstan to the north, by China and Tajikistan to the south, and by Uzbekistan to the west. It is almost entirely mountainous with only 7% of the land area suitable for arable agriculture. This characteristic makes the livestock sector one of the strongest and most important sectors of its economy [1]. The Kyrgyz population values horses as a source of meat and milk but also uses them as a means of transport and for sport activities. Kyrgyzstan has a total population of 433,000 horses, but numbers may be higher than the official figures [2]. The country is among the 10 biggest producers of horse meat with 22,000 t, approximately half of which is used for the farmers' own consumption [2]. The majority of horse farms in Kyrgyzstan are small and family owned, housing less than 10 animals

* Corresponding author at: Armando Mario Damiani, Área de Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Parque General San Martín S/N, 5500 Mendoza, Argentina.
E-mail address: amdamiani9@gmail.com (A.M. Damiani).

0737-0806/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.12.008

[1]. Consequently, mare reproductive failure leading to late-term abortion, stillbirth, or neonatal foal dead (together referred to as late fetal loss–LFL) results in a loss of milk and meat and direct economic losses. Late fetal loss is a major problem for horse farmers and breeders in the country, and increasing numbers of cases have been reported to the health authorities in the last years. Late fetal loss may be caused by noninfectious and infectious causes, with equine herpesvirus type 1 (EHV-1), equine arteritis virus (EAV), and, much less commonly, equine herpesvirus type 4 (EHV-4) being associated with the syndrome [3]. Ascending placentitis leads similarly to abortion or the birth of premature and weakened foals [4].

The present study aimed to investigate the role of important abortigenic infectious agents in LFL for the first time in the country.

2. Materials and Methods

2.1. Sample Collection

A total of 221 blood samples and 149 nasal swab specimens were collected from 221 mares in 42 farms which had experienced LFL within a year before sampling. Farms located in Issyk-Kul region, Chui region, and city of Bishkek were sampled between January–May 2013 (40 farms) and February–May 2014 (2 farms) (Fig. 1 and Table 1). Three sport horse and 39 family-owned (for dairy and meat production) farms were included in this screening. Sampled mares were from 3 to 15 years of age (median age: 5 years). Nasal swabs were placed in 2 mL of transport medium (phosphate-buffered saline containing 100 U/mL penicillin and 100 μg/mL streptomycin) and kept cold during transport to the laboratory. At the time of sampling, special attention was given to body condition and presence of nasal or vaginal discharge. Upon arrival in the laboratory, samples were appropriately aliquoted and kept frozen. Tissue samples (consisting in lung and/or placenta for every case plus kidney, liver, spleen, or brain in some cases) were available from 4 late-term aborted fetuses, 2 stillbirths, and 11 dead neonatal foals (Tables 1 and 2). These samples were obtained from necropsies taken place from September 2012 to March 2013 (16 cases) and February 2014 (1 case). Equine herpesvirus type 1 vaccination was never practiced in the country before and during the sampling period.

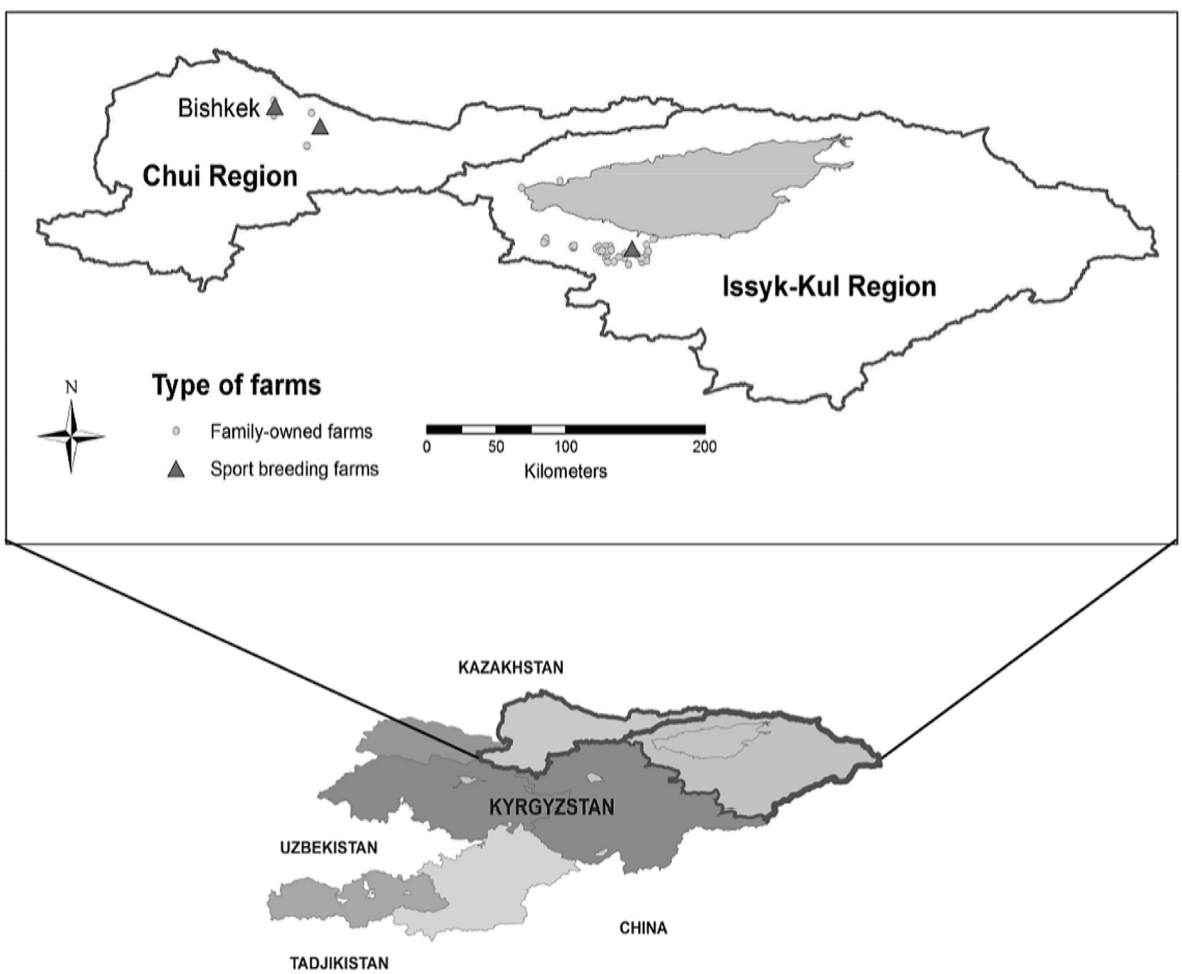

Fig. 1. Kyrgyzstan regions and location of farms sampled during the survey.

Table 1
Distribution of the samples collected during the study.

Type	Issyk-Kul Region		Chui Region		City of Bishkek		Total
	Dairy/ Meat	Sport	Dairy/ Meat	Sport	Dairy/ Meat	Sport	
Farms	35	1	2	1	2	1	42
Blood	149	21	19	22	6	4	221
Nasal swabs	117	0	10	12	6	4	149
Necropsied cases	4	1	6	2	3	1	17

2.2. Sample Processing and Analysis

Tissues (300–500 mg each) were homogenized with sterile sea sand using a mortar and pestle. Homogenates were suspended in 2 mL of Eagles's minimal essential medium with Earle's salts (Biochrom GmbH, Berlin, Germany) supplemented with 5% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Tissue debris was removed by low-speed centrifugation. One-hundred microliters of the supernatants was used for cocultivation with suspensions of rabbit kidney (RK-13) or horse dermal fibroblast (NBL-6) cells in 12-well plates for virus isolation. Nasal swabs in transport medium were vortexed, centrifuged at 6,000 rcf for 5 minutes, and 100 μL of supernatants used for cocultures as described previously. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and observed daily for cytopathic effect. Viral DNA and RNA were extracted using the RTP DNA/RNA Virus Mini Kit (Stratec Molecular GmbH, Berlin, Germany) from 200 μL of processed samples (swabs and homogenates) according to the manufacturer's instructions. Equine herpesvirus type 1, EHV-4, and EAV real-time PCRs (qPCR) were performed with the Applied Biosystems 7500 FAST real-time PCR system (Life Technologies, CA) using primers and probes described previously [5–7]. The protocols included an initial reverse transcription step at 48°C for 10 minutes (only for EAV) and a denaturation step at 95°C for 2 minutes followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. The total volume per well was 20 μL, including 10 μL of either SensiFAST Probe Lo-ROX mix (for EHV-1 and EHV-4) or SensiFAST Probe Lo-ROX one-step mix (for EAV–both kits from Bioline, London, UK), 450 nM of each forward and reverse primers, 100 nM probe, 5 μL of extracted nucleic acid, 0.2 μL of reverse transcriptase (for EAV), and nuclease-free water. Positive and negative controls were systematically included in every extraction/reaction.

For clustering of EHV-1 isolates, sequencing of the ORF68 region [8] was performed as described [9]. A hierarchical cluster analysis was carried out with the MEGA 5 software, using the unweighted pair group method with arithmetic mean. To investigate to which biotype (neuro- or nonneuropathogenic) the isolated strain belonged, an ORF30 PCR using primers forward (5′-GTGGACGGTACCCC-GGAC-3′) and reverse (5′-GTGGGGATT CGCGCCCTCACC-3′)

Table 2
Summary of the results collected during the investigation of 42 farms with LFL.

Samples Investigated			Serologic or Virological Results			Bacteriological Results[a]
			EHV-1	EHV-4	EAV	
221 sera[b]			192 had titers of 1:4 to 1:32 (median 1:8)	176 had titers of 1:4 to 1:32 (median 1:8)	<1:4 but 7 sera from one farm (1:32 to 1:512)	
149 nasal swabs			Neg	Neg but one	Neg	
	Late abortion	Lung, liver, spleen, placenta, kidney, brain	Neg	Neg	Neg	Neg
	Late abortion[c]	Lung, liver, spleen, placenta, kidney	Neg	Neg	Neg	Neg
	Stillbirth[c]	Lung, spleen	Neg	Neg	Neg	Neg
	Late abortion	Lung, placenta	Pos	Neg	Neg	Neg
	Late abortion	Lung, spleen	Neg	Neg	Neg	*E. coli*
	Neonatal dead	Lung, liver	Neg	Neg	Neg	Neg
	Stillbirth	Lung, liver, kidney	Neg	Neg	Neg	*E. coli/Enterococcus* sp.
	Neonatal dead	Lung, brain	Neg	Neg	Neg	*E. coli/Enterococcus* sp.
42 tissues from 17 LFL	Neonatal dead	Placenta	Neg	Neg	Neg	Neg
	Neonatal dead	Placenta	Neg	Neg	Neg	Neg
	Neonatal dead	Placenta	Neg	Neg	Neg	Neg
	Neonatal dead	Brain, lung	Neg	Neg	Neg	*E. coli/Enterococcus* sp./*Streptococcus equi* ssp. *zooepidermicus*
	Neonatal dead	Brain, lung	Neg	Neg	Neg	*E. coli*
	Neonatal dead	Lung, liver	Neg	Neg	Neg	Neg
	Neonatal dead	Lung, spleen	Neg	Neg	Neg	*E. coli*/*S. equi* ssp. *zooepidermicus*
	Neonatal dead	Brain, liver, lung	Neg	Neg	Neg	Neg
	Neonatal dead	Lung, kidney, brain, liver	Neg	Neg	Neg	Neg

Abbreviations: EAV, equine arteritis virus; EHV-1, equine herpesvirus type 1; EHV-4, equine herpesvirus type 4; LFL, late fetal loss; neg, negative; pos, positive.
[a] Lung and/or placenta tissues were investigated.
[b] 37 negatives and 3 positives for Dourine.
[c] Two cases collected at the EAV-positive farm.

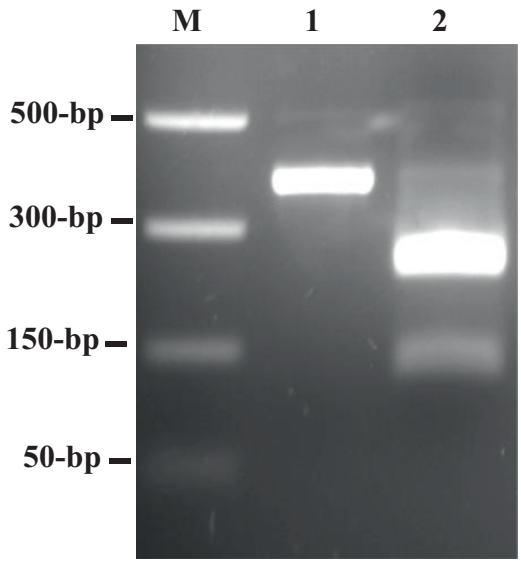

Fig. 2. 2% agarose gel electrophoresis of *Sal*I-treated ORF30 PCR product using EHV-1 KG14_1 (lane 1) and OH03 (neuropathogenic control, lane 2) as templates. EHV-1, equine herpesvirus type 1.

followed by *Sal*I digestion (Fig. 2) of the amplified product was performed as described [9]. An A to G substitution present in neuropathogenic EHV-1 variants at position 2254 of ORF30 introduces a *Sal*I site. Nonneuropathogenic strains remain uncut, whereas neuropathogenic strains are cut into two fragments of 247 and 133 bp. The PCR product was subsequently sequenced for confirmation. To exclude laboratory contaminations, an ORF1-ORF2 PCR using primers forward 5′-CCTTTTGGCTCTGGGTATTT-3′ and reverse 5′-ACGGCGGAAACCTAGGCGATGTGT-3′ with the conditions described in the ORF30 PCR protocol was applied [9].

Serum antibody titers against EHV-1, EHV-4, and EAV were determined by serum neutralization tests (SNTs) as described in the OIE Technical Manual [10,11]. For EHV-1, duplicates of twofold serial dilutions of heat-inactivated sera prepared in 96-well plates were mixed with an equal volume of a virus solution containing 100 tissue culture infectious dose 50% per well of EHV-1 strain RacL11. Dilutions ranged from 1:4 to 1:512 after the addition of the virus. The serum-virus mixtures were incubated 1 hour at 37°C, and 3×10^4 RK-13 cells were added. Cytophatic effects were assessed after 3 days of incubation at 37°C by fixation with 3% formalin in phosphate buffered saline and crystal violet staining. Equine herpesvirus type 4 and EAV SNT were technically performed as described previously. Equine herpesvirus type 4 SNT was executed with strain T252 and NBL-6 cells, whereas EAV SNT was carried out with Bucyrus strain prepared in a suspension containing 10% of guinea-pig complement and RK-13 cells. The titer of each serum was determined as the reciprocal of the highest dilution at which the monolayer was intact in both of the duplicate wells. Titers of $\geq$1:4 were considered as a positive result.

Routine aerobic culture of tissues samples was conducted at the Institute of Microbiology and Epizootics at Freie Universtiaet Berlin, whereas serum samples were sent to the German National Reference center for Dourine at the Friedrich-Loeffler-Institut of Jena to detect complement-fixing antibodies against *Trypanosoma equiperdum* [12].

3. Results

Findings are summarized in Table 2. Clinical examination at the time of sampling revealed neither nasal nor vaginal discharge in any animal of the sampled population. Body conditions of animals were considered average, except for one farm in Issyk-Kul region where the overall status of all sampled horses was regarded as poor. Tissue samples taken from all but one LFL cases resulted in negative outcomes by virus isolation and qPCR examination for EHV-1, EHV-4, and EAV. However, placental and fetal lung tissues from a late-term abortion case that occurred in a mixed sport-breeding farm from Issyk-Kul region tested EHV-1 positive by both virus isolation and PCR. Characteristic EHV-1 cytopathic effect (focal rounding of cells and cell detachment) was seen in cultures of RK-13 and NBL-6 cells. Negative results were obtained in all but one (EHV-4 positive by qPCR, Ct of 31) nasal swabs from the investigated mares. The isolated EHV-1 strain, named KG14_1, was characterized as “nonneurologic” (Pol A_{2254}) by enzyme restriction digestion (Fig. 2) and direct sequence analysis (GenBank Acc. No KU557665) of ORF30 PCR product. Phylogenetic analysis using the 631-bp sequenced ORF68 genomic region clustered EHV-1 strain KG14_1 into group 1 + 2 (Fig. 3). Analysis of the submitted ORF68 nucleotide sequence revealed the presence of a homopolymeric tract of 7 guanine (G) residues (see GenBank Acc. No KU557664). The ORF1_ORF2 PCR, which enables us to exclude cross-contamination, produced a 1,596-bp band using EHV-1 KG14_1 DNA as template, whereas EHV-1 strain RacL11 DNA (our routinely used laboratory strain) showed a distinct 313-bp PCR product (data not shown).

Equine herpesvirus type 1 and EHV-4 seropositivity was 87% (192 out of 221) and 80% (176 out of 221), respectively, of all mares tested. Low serum neutralizing antibody titers of 1:4 to 1:32 (median 1:8) against both viruses were detected in mares, a finding that suggests no implication of either virus in the abortion cases. On the other hand, surprisingly, high antibody titers in the range from 1:32 to 1:512 (median 1:256) were detected against EAV in serum samples of all seven mares on a sport horse farm located in the Chui region (Table 2).

Aerobic bacterial culture of lung and/or placenta tissues from 17 cases revealed the presence of *Escherichia coli* (one late abortion, one stillbirth, and four neonatal foal death cases), *Enterococcus* sp. (one stillbirth and two neonatal foal death cases), and *Streptococcus equi* ssp. *zooepidermicus* (two neonatal foal death cases). Complement-fixing antibody titers of 1:10, 1:640, and 1:1,280 against *Trypanosoma equiperdum* were detected in 3 out of 40 analyzed mares.

4. Discussion

Equine herpesvirus type 1 and EAV are considered the two major viral causes of LFL [3], with the former being

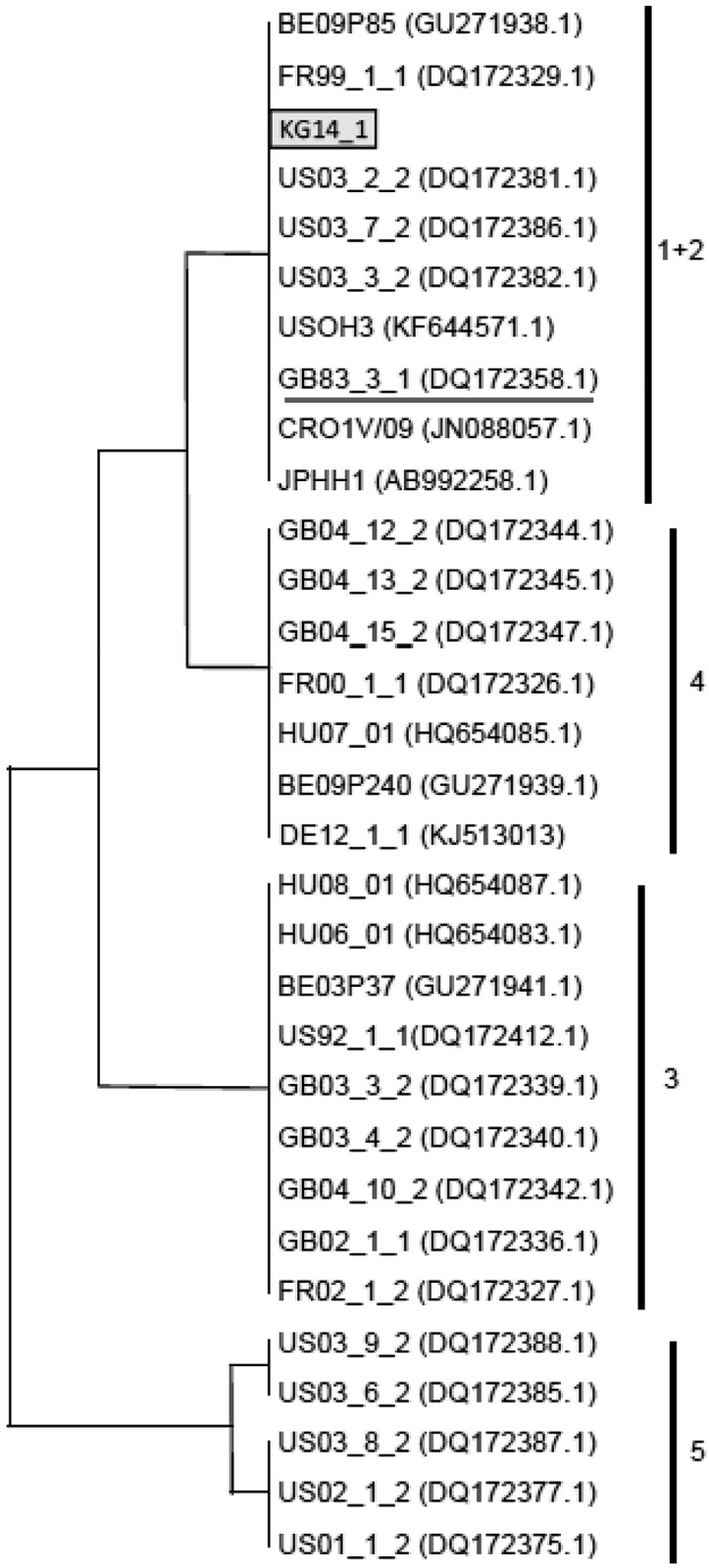

Fig. 3. Phylogenetic tree constructed by the UPGMA method using partial EHV-1 ORF68 sequences. Groups are identified according to Nugent et al (2006). The first two letters indicate origin of the isolate (US = United States; GB = Great Britain; HU = Hungary; CR = Croazia; DE = Germany; FR = France; BE = Belgium; JP = Japan). The Kyrgyzian isolate KG14_1 is highlighted. GenBank accession numbers are indicated. A representative group 1 strain included in the analysis is underlined. EHV-1, equine herpesvirus type 1; UPGMA, unweighted pair group method with arithmetic mean.

considered a frequent source of pregnancy loss in horses worldwide [13–19]. Equine herpesvirus type 1 outbreaks commonly occur in the last trimester of pregnancy and may result in abortions storms, with abortion rates of up to 80% [20]. Equine arteritis virus–induced abortions can occur at any time between 3 and 10 months of gestation, with abortion rates during field outbreaks varying from 10% to 70% depending on the virus strain [21–23]. Although not common, EAV has been described as a cause of neonatal foal death [24]. Equine herpesvirus type 4, which shares high degrees of genetic and antigenic similarity with EHV-1, has also been implicated in late-term abortions in mares, although rarely [25]. Serologic evidence of exposure to EHV-1 and EHV-4 in the horse population studies was expected, as both viruses are distributed worldwide [26]. Seroprevalences of 87% and 80% were found for EHV-1 and EHV-4, respectively. Positivity rates were low (median neutralizing antibody titer of 1:8), likely reflecting the endemic nature of both viruses in the Kyrgyz horse population, rather than the two agents being the cause for the observed LFL in the investigated farms. If the two viruses had been the source of abortion that occurred during the last pregnancy, we would have expected higher neutralizing antibody titers in the sampled population. After an EHV-1 outbreak, serum neutralizing antibody titers usually rise substantially and remain at relatively constant levels for months [9]. Supporting the serum neutralization results, neither PCR amplification nor virus isolation was successful in tissues from late-term aborted fetuses, stillbirths, or neonatal foals deaths collected before starting serum sampling in April 2013. Surprisingly, given the lack of evidence for EHV-1 as the causal agent of LFL in 2013, a late-term abortion case that occurred in February 2014 tested positive for EHV-1. The sports horse–breeding farm located in the Issyk-Kul region, which holds 27 mares, was reported to have experienced 9 LFL between December 2013 and February 2014. Neither tissues nor blood from the other eight cases were submitted for laboratory investigation.

Laboratory cross-contamination with a routinely used EHV-1 RacL11 strain was excluded by applying a discriminatory PCR. Compared to field strains, RacL11 possesses a deletion of 1,283 bp resulting in the absence of the majority of ORF1 and the complete ORF2 [27]. Molecular characterization revealed a non-neuropathogenic, or abortigenic, genotype in the isolated EHV-1 strain KG14_1. Indeed, signs of neurologic manifestation of infection were not reported after or before the case. The A_{2254} genotype was shown to be predominant in nonneurologic EHV-1 abortion outbreaks worldwide [28–32]. When a phylogenetic tree was constructed based on a variable ORF68 region, EHV-1 KG14_1 was allocated into group 1 + 2 [8], represented by European, Japanese, and American isolates of EHV-1. Groups 1 and 2 appear always superimposed on ORF68-based evolutionary trees [8,9,33,34], and a detailed analysis of the determined sequence is required to allocate isolates precisely into group 1 or 2 [8]. The Kyrgyz isolate possesses a homopolymeric tract of seven G residues characteristic of all but group 1 (which contains eight, six or nine G residues) indicating KG14_1 strain as a group 2 isolate. It is therefore predicted that EHV-1 KG14_1 codes for a truncated version of ORF68 protein as the majority of EHV-1 strains. Within group 2, relatively recent isolates from Europe and the United States are clustered together. Thoroughbred racing activities in Kyrgyzstan and other Central Asian countries as Kazakhstan have increased in the last years [35], with horses being imported to the country for breeding purposes. The EHV-1 positive case occurred in a sports horse–breeding farm and virus was isolated from placenta of an aborting Thoroughbred mare and lung of her lost foal. Whether the breeding farm imported mares from Europe or United States is unknown.

High neutralizing antibody titers against EAV (median 1:256) were detected in all seven mares on a breeding farm in Chui region indicating exposure to EAV. The farm breeds sports horses and houses 25 mares and 2 stallions. Some animals were purchased abroad and the owner was reluctant to give further information. As vaccination against EAV is not common practice in Kyrgyzstan, antibody titers against the virus may be the result of either vaccination/infection abroad or past/recent infection on site. No signs indicative for active EAV infection were recorded during sampling. Tissues from two LFLs that occurred early in the year were available for virological examination and tested negative for EAV. We consequently assume that EAV was not implicated in those recent cases. The presence of EAV carrier stallions in the farm should be investigated, as those are the source for outbreaks of the disease when used for insemination of immunologically naïve mares [22,36].

Although we focused our interest on viral causes of abortion, serologic screening for Dourine was also carried out. Dourine screening was done in five mares of a farm in which sudden deaths, preceded by the loss of coordination in two foals, were reported by the owner. Visual examination of the mares revealed poor body conditions and a single edematous patch of 5 cm in a foal, symptoms suggestive of Dourine infection [12,37]. No tissues from LFL cases were available for examination in this farm. The testing was also applied randomly to 35 mares from 18 farms included in the study. Although clinical signs compatible with the disease were neither reported nor detected at the time of sampling, three mares from different farms resulted positive for Dourine with titers of 1:10, 1:640, and 1:1,280. However, the specificity of the dourine complement fixation test is not well characterized, with cross-reactivity observed due to the presence of other trypanosomes, for example, *Trypanosoma brucei* and *Trypanosoma evansi*. Thus, positive results in the absence of clinical disease need to be interpreted with caution. The latest Dourine outbreak in the country was reported to the World Animal Health in 2002.

In a further attempt to identify the origin of LFL, bacteriologic examination of lung and placenta tissue samples taken from aborted fetuses, stillbirths, and dead neonatal foals was performed. Routine aerobic culture revealed the presence of *E. coli* and/or *Streptococcus equi* (subsp. *zooepidemicus*) in the lungs of four dead

neonatal foals. These microorganisms are frequently associated with placentitis and found in foal tissues from fatal cases of neonatal septicemia [38,39]. Bacterial placentitis is a common cause of late-term abortion and perinatal complications in mares and poses a significant threat to fetal and neonatal viability. A work conducted in Australia clearly demonstrated that ultrasound monitoring and treatment (antibiotics and different combinations of anti-inflammatory drugs and altrenogest) of high-risk mares improves fetal and neonatal viability [40].

5. Conclusion

To the authors' knowledge, this is the first report with the aim to investigate for causes of LFL in Kyrgyzstan. Evidence of EHV-1 as a causal agent of a late abortion case in a sports horse–breeding farm is provided. An investigation with the purpose to detect placental abnormalities as the result of bacterial infection in pregnant mares owned to breeding farms for meat and/or milk production will therefore be conducted in the country in the coming years.

Acknowledgments

Special thanks to Tine Leiskau, Sebastian Bischofberger, Krutskaya Ekaterina, Saadanov Iskender, Isakeev Mayrambek, and Tolubaeva Mayram for their excellent technical assistance.

References

[1] World Bank. Kyrgyz Republic: livestock sector review-embracing the new challenges. Available at: http://documents.worldbank.org/curated/en/497831468266648728/Kyrgyz-Republic-livestock-sector-review-embracing-the-new-challenges; 2007 [accessed 06.09.16].
[2] FAOSTAT. Food and Agriculture Organization of the United Nations, Statistical Databases. Available at, http://faostat.fao.org; 2014 [accessed 12.09.16].
[3] Bazanów BA, Jackulak NA, Fracka AB, Staroniewicz ZM. Abortigenic viruses in horses. Equine Vet Educ 2014;26:48–55.
[4] Cummins C, Carrington S, Fitzpatrick E, Duggan V. Ascending placentitis in the mare: a review. Irish Vet J 2008;61:307–13.
[5] Balasuriya UB, Leutenegger CM, Topol JB, McCollum WH, Timoney PJ, MacLachlan NJ. Detection of equine arteritis virus by real-time TaqMan reverse transcription-PCR assay. J Virol Methods 2002;101:21–8.
[6] Hussey SB, Clark R, Lunn KF, Breathnach C, Soboll G, Whalley JM, Lunn DP. Detection and quantification of equine herpesvirus-1 viremia and nasal shedding by real-time polymerase chain reaction. J Vet Diagn Invest 2006;18:335–42.
[7] Pusterla N, Leutenegger CM, Wilson WD, Watson JL, Ferraro GL, Madigan JE. Equine herpesvirus-4 kinetics in peripheral blood leukocytes and nasopharyngeal secretions in foals using quantitative real-time TaqMan PCR. J Vet Diagn Invest 2005;17:578–81.
[8] Nugent J, Birch-Machin I, Smith KC, Mumford JA, Swann JR, Newton R, et al. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. J Virol 2006;80:4047–60.
[9] Damiani AM, de Vries M, Reimers G, Winkler S, Osterrieder N. A severe equine herpesvirus type 1 (EHV-1) abortion outbreak caused by a neuropathogenic strain at a breeding farm in northern Germany. Vet Microbiol 2014;172:555–62.
[10] OIE. Equine rhinopneumonitis. In: Manual of diagnostic tests and vaccines for terrestrial animals. 7th edn. France: World Organization for Animal Health (OIE); 2012. p. 889–98.
[11] OIE. Equine viral arteritis. In: Manual of diagnostic tests and vaccines for terrestrial animals. 7th edn. France: World Organization for Animal Health (OIE); 2012. p. 899–912.
[12] OIE. Dourine. In: Manual of diagnostic tests and vaccines for terrestrial animals. 7th edn. France: World Organization for Animal Health (OIE); 2012. p. 838–45.
[13] Matsumura T, Sugiura T, Imagawa H, Fukunaga Y, Kamada M. Epizootiological aspects of type 1 and type 4 equine herpesvirus infections among horse populations. J Vet Med Sci 1992;54:207–11.
[14] Giles RC, Donahue JM, Hong CB, Tuttle PA, Petrites-Murphy MB, Poonacha KB, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). J Am Vet Med Assoc 1993;203:1170–5.
[15] Tengelsen LA, Yamini B, Mullaney TP, Bell TG, Render JA, Patterson JS, et al. A 12-year retrospective study of equine abortion in Michigan. J Vet Diagn Invest 1997;9:303–6.
[16] Butler C, Werners A, Newton R. 2011: Surveillance of infectious and non-infectious causes of equine abortion in the UK: 2006-2011. In: DEFRA/AHT/BEVA Equine Quarterly Disease Surveillance Report 7(1). Available at: https://www.aht.org.uk/skins/Default/pdfs/equine_vol7_1.pdf. [accessed 12.09.16].
[17] Laugier C, Foucher N, Sevin C, Leon A, Tapprest J. A 24-year retrospective study of equine abortion in Normandy (France). J Equine Vet Sci 2011;31:116–23.
[18] Marenzoni ML, Lepri E, Casagrande Proietti P, Bietta A, Coletti M, Timoney PJ. Causes of equine abortion, stillbirth and neonatal death in central Italy. Vet Rec 2012;170:262.
[19] Smith KC, Blunden AS, Whitwell KE, Dunn KA, Wales AD. A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. Equine Vet J 2003;35:496–501.
[20] Allen GP, Kydd JH, Slater JD, Smith KC. Equid herpesvirus 1 and equid herpesvirus 4 infections. In: Coetzer JAW, Tustin RC, editors. Infectious diseases of livestock. 1st edn. Newmarket: Oxford University Press; 2004. p. 829–59.
[21] Timoney PJ, McCollum WH. Equine viral arteritis. Vet Clin North Am Equine Pract 1993;9:295–309.
[22] Pronost S, Pitel PH, Miszczak F, Legrand L, Marcillaud-Pitel C, Hamon M, et al. Description of the first recorded major occurrence of equine viral arteritis in France. Equine Vet J 2010;42:713–20.
[23] Balasuriya UB, Go YY, MacLachlan NJ. Equine arteritis virus. Vet Microbiol 2013;167:93–122.
[24] Gryspeerdt A, Chiers K, Govaere J, Vercauteren G, Ducatelle R, Van de Walle GR, et al. Neonatal foal death due to infection with equine arteritis virus in Belgium. Vlaams Diergen Tijds 2009;78:189–93.
[25] Gerst S, Borchers K, Gower SM, Smith KC. Detection of EHV-1 and EHV-4 in placental sections of naturally occurring EHV-1- and EHV-4-related abortions in the UK: use of the placenta in diagnosis. Equine Vet J 2003;35:430–3.
[26] Foote CE, Love DN, Gilkerson JR, Wellington JE, Whalley JM. EHV-1 and EHV-4 infection in vaccinated mares and their foals. Vet Immunol Immunopathol 2006;15:41–6.
[27] Ma G, Feineis S, Osterrieder N, Van de Walle GR. Identification and characterization of equine herpesvirus type 1 pUL56 and its role in virus-induced downregulation of major histocompatibility complex class I. J Virol 2012;86:3554–63.
[28] Perkins GA, Goodman LB, Tsujimura K, Van de Walle GR, Kim SG, Dubovi EJ, et al. Investigation of the prevalence of neurologic equine herpes virus type 1 (EHV-1) in a 23-year retrospective analysis (1984-2007). Vet Microbiol 2009;139:375–8.
[29] Vissani MA, Becerra ML, Olguín Perglione C, Tordoya MS, Miño S, Barrandeguy M. Neuropathogenic and non-neuropathogenic genotypes of Equid Herpesvirus type 1 in Argentina. Vet Microbiol 2009;139:361–4.
[30] Pronost S, Léon A, Legrand L, Fortier C, Miszczak F, Freymuth F, et al. Neuropathogenic and non-neuropathogenic variants of equine herpesvirus 1 in France. Vet Microbiol 2010;145:329–33.
[31] Fritsche AK, Borchers K. Detection of neuropathogenic strains of Equid Herpesvirus 1 (EHV-1) associated with abortions in Germany. Vet Microbiol 2011;147:176–80.
[32] Stasiak K, Rola J, Ploszay G, Socha W, Zmudzinski JF. Detection of the neuropathogenic variant of equine herpesvirus 1 associated with abortions in mares in Poland. BMC Vet Res 2015;11:102.
[33] Barbić L, Lojkić I, Stevanović V, Bedeković T, Starešina V, Lemo N, Lojkić M, Madić J. Two outbreaks of neuropathogenic equine herpesvirus type 1 with breed-dependent clinical signs. Vet Rec 2012;170:227.
[34] Negussie H, Gizaw D, Tessema TS, Nauwynck HJ. Equine Herpesvirus-1 myeloencephalopathy, an emerging threat of working equids in Ethiopia. Transbound Emerg Dis 2015. http://dx.doi.org/10.1111/tbed.12377.

[35] Tolenovich TN, Makhatovich MB, Zhaleluly IZ. Current state of Kazakhstan Thoroughbred Horsebreeding. Biosci Biotechnol Res Asia 2015;12:1357–62.
[36] Miszczak F, Legrand L, Balasuriya U, Ferry-Abitbol B, Zhang J, Hans A, et al. Emergence of novel equine arteritis virus (EAV) variants during persistent infection in the stallion: origin of the 2007 French EAV outbreak was linked to an EAV strain present in the semen of a persistently infected carrier stallion. Virology 2012;20:165–74.
[37] Scacchia M, Cammà C, Di Francesco G, Di Provvido A, Giunta R, Luciani M, et al. A clinical case of dourine in an outbreak in Italy. Vet Ital 2011;47:473–5.
[38] Hong CB, Donahue JM, Giles Jr R, Petrites-Murphy MB, Poonacha KB, Roberts AW, et al. Etiology and pathology of equine placentitis. J Vet Diagn Invest 1993;5:56–63.
[39] McKenzie III HC, Furr MO. Equine Neonatal Sepsis: the pathophysiology of severe inflammation and infection in the equine neonate. Comp Cont Educ Pract 2001;23:661–72.
[40] Carrick JB. Placentitis in horses, a major cause of late term foetal loss. Rural Industries Research and Development Corporation, Project No. PRJ-000744, Publication No. 10/156, Australia. Available at, https://rirdc.infoservices.com.au/downloads/10-156; 2010 [accessed 12.09.16].