

Contents

List of Tables	9
List of Figures	11
1 Introduction	15
2 Review of literatures	16
2.1 Taxonomy of Equid Herpesviruses	16
2.2 Equid Herpesvirus 1, 4 and 9	16
2.2.1 Morphological structure	16
2.2.2 Genome structure	17
2.2.3 The DNA polymerase (open reading frame 30)	17
2.2.4 Latency	18
2.2.5 Epidemiology	19
2.2.6 Clinical signs	20
2.2.7 Treatment	21
2.2.8 Prophylaxis and Management	22
2.2.9 Pathogenesis	22
2.2.10 Detection methods	27
3 Objective of the thesis	29
4 Material and methods	30
4.1 Proof of material	30
4.1.1 Chemicals and solutions	30
4.1.2 Materials	31
4.1.3 Equipment	31
4.2 EHV-1 and -4 reference strains	33
4.2.1 EHV-1 reference strains	33
4.2.2 EHV-4 reference strains	33
4.3 Wild animal and cattle EHV strains	34
4.3.1 Wild animal EHV strains	34
4.3.2 Cattle EHV-1 strains	35
4.4 Sample origin	35
4.4.1 Single abortion cases	36
4.4.2 Abortion outbreaks in Germany	36
4.4.3 Abortion outbreak stud farm 1	36
4.4.4 Abortion outbreak stud farm 2	37
4.4.5 Abortion outbreak stud farm 3	37
4.4.6 Abortion outbreak stud farm 4	38
4.4.7 Neurological cases	39
4.5 Cell cultures	40
4.5.1 Cell passaging	40

4.6	Serological tests	41
4.6.1	Neutralization test (NT)	41
4.6.2	Immunofluorescence assay (IFA)	42
4.7	PBMC isolation from citrated blood	43
4.8	DNA preparation	44
4.8.1	DNA preparation from tissue samples and PBMCs	44
4.8.2	Viral DNA preparation from virus stocks, infected cell culture supernatants, nasal swabs, lung fluids, amniotic fluid and semen	44
4.9	Analytical gel electrophoresis	45
4.10	Determining the molecular masses and concentration of DNA	46
4.10.1	Gel electrophoresis	46
4.10.2	Photometry	46
4.11	Polymerase chain reaction (PCR)	46
4.11.1	ORF 30 nested PCR	47
4.11.2	Evaluation of ORF 30 nested PCR	49
4.11.3	Sensitivity of the ORF 30 nested PCR	50
4.12	Restriction enzyme analysis (REA)	50
4.12.1	REA <i>Sal</i> I of ORF 30 nested PCR products	50
4.12.2	Evaluation of the REA <i>Sal</i> I digestion	51
4.13	Sequence analysis	51
4.14	Statistics	52

5 Results 53

5.1	Establishment and evaluation of the ORF 30 nested PCR	53
5.1.1	Establishment of the ORF 30 nested PCR with selected EHV-1/-4 reference strains	54
5.1.2	Sensitivity of the ORF 30 nested PCR	55
5.2	Test of the restriction enzyme analysis <i>Sal</i> I with the ORF 30 fragments of the selected EHV-1 reference strains	56
5.3	Review of the ORF 30 fragments of the selected EHV-1 reference strains by sequencing	58
5.3.1	Chromatograms of the ORF 30 fragments of the selected EHV-1 reference strains	59
5.4	Occurrence of the non-neuropathogenic versus the neuropathogenic genotype in EHV-1 reference strains and wild animal and cattle strains	59
5.4.1	EHV-1 reference strains	61
5.4.2	Wild animals and cattle strains	62
5.5	Occurrence of the neuropathogenic EHV-1 genotype in CNS cases	64
5.6	Sample character of the EHV-1 abortion cases	65
5.7	Occurrence of the non-neuropathogenic versus the neuropathogenic genotype in EHV-1 abortion cases	65
5.7.1	Investigation of abortion strains by ORF 30 nested PCR	65
5.7.2	Investigation of the ORF 30 amplicons by restriction enzyme analysis <i>Sal</i> I	67
5.7.3	Sequencing of selected ORF 30 amplicons	74

5.7.4	Additional mutation in the ORF 30 amplicons from abortion cases	78
5.7.5	Stud farm 4: Detection of viral DNA in PBMC and determination of the genotype	78
5.8	Serological studies	79
5.8.1	Stud farm 3 with abortions and neurological signs	79
5.8.2	Stud farm 4 with an abortion outbreak	83
6	Discussion	85
6.1	Detection of the non-neuropathogenic versus the neuropathogenic EHV-1 genotype in abortion cases in Germany	85
6.2	EHV-1 in stallions	89
6.3	Additional nucleotide exchanges in the ORF 30 in Germany	90
6.3.1	Nucleotide exchange at position 2258	90
6.3.2	Nucleotide exchange at position 2269	91
6.4	Detection of the neuropathogenic genotype in cattle and archived wild equid strains	91
6.4.1	Nucleotide exchange at position 2262	92
6.5	Detection of the neuropathogenic EHV-1 genotype in neurological cases in Germany	92
6.6	Serological and molecular biological detection of EHV-1 in abortion outbreaks on two stud farms	93
6.6.1	Stud farm 3	93
6.6.2	Stud farm 4	94
7	Summary	96
8	Zusammenfassung	97
9	Bibliography	98
10	Annex	110
10.1	Reference strains	110
10.1.1	Origin of the EHV-1/-4 reference strains and wild animal strains, sample character, EHV-1/-4 classification and processed sample character	110
10.1.2	Results of the ORF 30 nested PCR, <i>SalI</i> restriction enzyme analysis and sequencing of the reference strains and wild animal strains	111
10.1.3	Sequences of the reference strains and wild animal strains	111
10.2	Abortion cases	112
10.2.1	Origin of the 67 EHV-1 abortion cases, sample character, processed sam- ple character, ORF 30 nested PCR and <i>Sal I</i> restriction enzyme results. Selection of the ORF 30 amplicons for sequencing	112
10.2.2	Sequences of the abortion strains from 1987 to 2009	119
10.3	Neurological cases	121
10.3.1	Results of the ORF 30 nested PCR, <i>SalI</i> restriction enzyme analysis and selection of the ORF 30 amplicons for sequencing of neurological cases . . .	121
10.3.2	Sequences of the neurological EHV strains	121
10.4	Report of the clinical signs of the sampled horses from stud farm 3	121

10.5 Serology	122
10.5.1 Stud farm 3: EHV-1/-4 serum neutralization and immunofluorescence assay	122
10.5.2 Stud farm 4: EHV-1/-4 neutralization test	123
10.6 List of own publications	124
10.7 Danksagung	125
10.8 Selbständigkeitserklärung	126