## Table of contents

Abstract	ı
Zusammenfassung l	ı
Abbreviations II	I
Table of contentsVI	ı
List of Figures	K
1. Introduction	1
1.1 Epidemiology and pathology of influenza C virus	1
1.2 Evolutionary relationship of influenza C with other viruses	2
1.3 Viral particle and genome structure of influenza C virus	3
1.4 HEF protein of influenza C virus	5
1.4.1 Primary structure of HEF protein	7
1.4.2 Crystal structure of the HEF protein	В
1.4.3 Co-and post-translational modifications of HEF	9
1.4.4 Regular arrangement of HEF spikes in virus particles	5
1.4.5 Receptor binding activity of HEF	6
1.4.6 Membrane fusion activity of HEF	7
1.4.7 Receptor hydrolysis (esterase) activity of HEF2	0
1.5 The other proteins of influenza C virus	2
1.6 Reverse genetics of influenza C virus	3
1.7 Why study Influenza C virus	4
2. Aim of the thesis	6
3. Materials 2	8
3.1 Kits	8
3.2 Bacteria and cells	8
3.3 Apparatuses	8
3.4 Enzymes and regents	0
3.5 Antibodies	1
4. Method	2
4.1 Maxi preparation of bidirectional plasmids for influenza C virus reverse genetics 3	2
4.2 Constructing pPMV plasmid with non-acylated HEF sequence by site-directed mutagenesis	3
4.3 Generation of mutant and wt influenza C viruses by reverse genetics	5

	4.4 Determination of infectious titers of viruses by TCID <sub>50</sub>	. 37
	4.5 Compare growth kinetics of wt and mutant strains	. 38
	4.6 Continuous passages of the mutant and wt strains	. 38
	4.7 Competitive growth of wt and mutant viruses	. 38
	4.8 Comparison of protein composition between wt and mutant viruses by SDS-PAGE	. 39
	4.9 Electron microscope of the viral particles	. 40
	4.10 Testing antibodies for metabolic labeling & immunoprecipitation	. 41
	4.11 Cell membrane transport kinetics of wt and non-acylated HEF	. 41
	4.12 Hemolysis assay of mutant influenza C virus with non-acylation HEF and wt virus $\boldsymbol{\theta}$	. 43
	4.13 pH dependent binding of fluorophore bis-ANS to wt and mutant strain	45
5.	Results	46
	5.1 Generation of mutant and wt influenza C viruses by reverse genetics	. 46
	5.2 Growth kinetics of wt and mutant strains	. 47
	5.3 Continuous passages of the mutant and wt strains	. 48
	5.4 Competitive growth of wt and mutant viruses	. 48
	5.5 Protein composition of wt and mutant viruses by SDS-PAGE	49
	5.6 Electron microscope of the viral particles	. 50
	5.7 Antibody selection for metabolic labeling & immunoprecipitation	. 53
	5.8 Transport of wt and non-acylation mutant HEF to the plasma membrane	. 54
	5.9 Hemolysis assays of wt and mutant influenza C virus	. 55
	5.10 Binding of fluorophore bis-ANS to wt and non-acylation HEF at low pH condition .	. 57
6.	Discussions	61
7.	References	64
8.	Supplemental material	. 73
9.	Acknowledgements	74
S	elbständigkeitserklarung	75