RESPIRATORY PATHOGENS IN THOROUGHBRED FOALS UP TO ONE YEAR OF AGE ON A STUD FARM IN SOUTH AFRICA

BY

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Submitted in partial fulfilment of the requirements for the degree of Master of Science (MSc) (Veterinary Science) in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa

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ABSTRACT

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The project was undertaken to monitor a group of 30 foals on a farm both clinically and microbiologically from birth until one year of age, to determine the aetiology of upper respiratory tract infections and to establish immune profiles of some of the known respiratory viral pathogens.

One to two months prior to the birth of their foals, blood for serology was collected from the mares. The same specimens were collected from the foals just after birth, prior to suckling and a day after suckling. Thereafter the foals were examined monthly for the presence of respiratory disease and specimens taken. The following specimens were collected from each foal: three nasopharyngeal swabs, (one for virus isolation, one for bacteria and fungus isolation, and one for mycoplasma isolation) and blood that was allowed to clot. Blood was collected in heparin from sick foals with elevated rectal temperatures. Virus isolation was done on roller tube cultures of equine embryonic lung (EEL), Vero cells and rabbit kidney 13 (RK13) cells. The bacteria (including mycoplasmas) and fungi were cultured from the swabs and identified using a variety of traditional methods. The serum neutralization test (SNT) was used to detect antibodies to equid herpesvirus 1 (EHV-1), equid herpesvirus 4 (EHV-4), equine rhinovirus 1 (ERV-1), equine rhinovirus 2 (ERV-2) and equine adenovirus 1 (EAdV-1). The complement fixation test (CFT) was used to detect antibodies to EHV-1 and EHV-4 and the haemagglutination inhibition test (HIT) antibodies to equine influenzavirus (EIV).

Only EHV-4 was cultured from the nasopharyngeal swabs of nine foals when they were 5 to 6 months of age and from one foal two months later. A wide variety of bacteria and fungi were cultured and it was established that coagulase-negative staphylococci, viridans streptococci, *Moraxella* spp. and *Flavobacterium* spp. predominated in most of the samples. Several potential bacterial pathogens were isolated but the most common were *Streptococcus equi* subsp. *zooepidemicus*, *Actinobacillus equuli* and *Staphylococcus aureus.*

Colostrum-derived antibodies were detected for all the viruses in all but two of the foals. It was found that the foals had similar or slightly higher titres than their mothers. The levels declined in direct proportion to what they initially were and were depleted by the time the foals were 2 to 7 months of age. Antibodies to natural infection was detected to EHV-4, ERV-2 and EAdV-1. A rise in antibody titres occurred when the foals were 5 to 6 months of age, two months later and when they were one year of age. Antibodies resulting from immunization was detected to EHV-1, EHV-4 and EIV.

It was established that the most important virus causing upper respiratory tract disease of the foals from 5 to 12 months of age was EHV-1 with EAdV-1 playing a minor role. These viruses caused repeated bouts of infection with a two to five months interval. *Streptococcus equi* subsp. *zooepidemicus* was considered to be the most important secondary pathogen. Prior to this period most of the foals were healthy with only a few suffering from upper respiratory disease. The aetiology was not determined in these cases,

but based on the bacteriology results, it was suspected that some of them were suffering from bacterial infections.

KEY WORDS: foals, infectious respiratory tract disease, equid herpesvirus 1, equid herpesvirus 4, equine adenovirus, equine rhinovirus 1, equine rhinovirus 2, equine influenzavirus, South Africa

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ABBREVIATIONS

ASPV	Acid-stable picornavirus
AHS	African horse sickness
AHSV	African horse sickness virus
AHV-1	Asinine herpesvirus 1
AHV-3	Asinine herpesvirus 3
ATCC	American Type Culture Collection
ATV	Trypsin-versene solution
BAL	Broncho-alveolar lavage
CF	Complement fixing
CFT	Complement fixation test
CID	Combined immunodeficiency disease
CO_2	Carbon dioxide
CPE	Cytopathic effect
°C	Degrees Celsius
DMSO	Dimethylsulphoxide
DVTD	Department of Veterinary Tropical Diseases
EAdV-1	Equine adenovirus 1
EAV	Equine arteritis virus
EEL	Equine embryonic lung
EEV	Equine encephalosis virus
EHV-1	Equid herpesvirus 1
EHV-2	Equid herpesvirus 2
EHV-4	Equid herpesvirus 4
EHV-5	Equid herpesvirus 5
EI	Equine influenza
EIV	Equine influenzavirus
EMEM	Eagles-minimum essential medium
ERV-1	Equine rhinovirus 1
ERV-2	Equine rhinovirus 2
ERV-3	Equine rhinovirus 3
EVA	Equine viral arteritis
FCS	Foetal calf serum
G	Gauge hypodermic needle
G	Gravitational force
GM	Gram-negative
GP	Gram-positive
H	Haemagglutinin
HI	Haemagglutination inhibiting antibodies
HIT	Haemagglutination inhibition test
IFAT	Immunofluorescent antibody test
kg	Kilogram
MBA	Microbiotic agar
mg	Milligram
MHC	The minimum haemolytic dose of complement
MHD	Minimum haemolytic dose
ml	Millilitres
MP	Matrix protein
N	Neuraminidase
NP	Nucleoprotein
PBS	Phosphate buffered saline
PBS-	Calcium and magnesium free phosphate buffered saline
PI-3	Parainfluenza-3 virus
SDA	Sabouraud's dextrose agar

SEM	Scanning electron microscopy
SN	Serum neutralizing
SNT	Serum neutralization test
SRBC	Sheep red blood cells
$TCID_{50}$	Tissue Culture Infective $Dose_{50}$
TEM	Transmission electron microscope
TSI	Triple sugar iron agar
TTA	Transtracheal aspirate/wash
VBS	Veronal buffered saline
µl	Microlitres
XLD	Lysine dextrose

TABLE OF CONTENTS

List of tables

List of figures

CHAPTER ONE

1.1 BACKGROUND

Managers of equine stud farms and veterinarians worldwide encounter a number of infectious respiratory problems, including pyrexia, purulent nasal discharges, persistent cough, pneumonia, and lung abscesses in foals and yearlings (Hoffman et al., 1999). Infectious respiratory disease can result in economic losses due to poor growth and performance, and occasional deaths, as well as costly therapy. It is considered to be an important cause of disease and mortality in one to six-month-old foals. In a survey done in 1991 in Texas, veterinarians considered it to be the cause of 19 % of fatalities and 22 % of disease in foals (Cohen, 1994). Certain respiratory viral infections, such as equid herpesviruses may result in latent carriers while *Streptococcus equi* subspecies *equi* may be harboured in the nasal cavity and guttural pouches in some horses that have recovered from these infections (Chanter, 1998). These short- and long-term carrier animals may act as a source of infection and thus trigger new epidemics. In the long-term, lower respiratory tract disease in foals has been associated with exercise-induced pulmonary haemorrhage and other performance disorders when they are mature (Bernard et al., 1991; Hoffman et al., 1999).

Although equine respiratory disease has been well studied in many countries of the world (Hoffman et al., 1999; Sherman et al., 1979; Sugiura et al., 1987), very little has been published on the aetiology and prevalence of respiratory disease in foals in South Africa.

In this study 30 foals were monitored both clinically and microbiologically from birth until one year of age, to determine the aetiology of upper respiratory tract infections and to establish immune profiles for some of the known respiratory viral agents.

1.2 STUDY OBJECTIVES

To study the viral, bacterial (including mycoplasmal infections) and fungal microflora of the upper respiratory tract in 30 foals on a Thoroughbred stud farm from birth to one year of age.

To determine the aetiology of upper respiratory tract disease in the same foals, when clinical evidence is presented.

To monitor the monthly antibody response from birth to weaning in the foals to 6 viruses that cause respiratory tract infections, namely equid herpesvirus 1 (EHV-1), equid herpesvirus 4 (EHV-4), equine rhinovirus 1 (ERV-1), equine rhinovirus 2 (ERV-2), equine adenovirus 1 (EAdV-1) and equine influenzavirus (EIV).

1.3 LITERATURE REVIEW

1.3.1 AETIOLOGY

Many viruses, bacteria and fungi cause or contribute to the development of respiratory disease in foals. These are listed in Table 1.

Table 1: Summary of infectious agents that are known to cause, or are suspected of causing respiratory disease in foals

VIRUSES	BACTERIA & FUNGI
Acid-stable picornavirus (ASPV) African horse sickness virus (AHSV) Equid herpesvirus 1 (EHV-1) Equid herpesvirus 2 (EHV-2) Equid herpesvirus 4 (EHV-4) Equid herpesvirus 5 (EHV-5) Equine adenovirus 1 (EAdV-1) Equine arteritis virus (EAV) Equine encephalosis virus (EEV) Equine influenzavirus (EIV) Equine parainfluenza virus 3 Equine reovirus Equine rhinovirus 1 (ERV-1) Equine rhinovirus 2 (ERV-2) Equine rhinovirus 3 (ERV-3)	*Acinetobacter* spp. *Actinobacillus equuli* *Actinobacillus* spp. *Bacteroides* spp. *Bordetella bronchiseptica* *Burkholderia (Pseudomo*nas) mallei *Clostridium* spp. *Escherichia coli* *Eubacterium* spp. *Fusobacterium* spp. *Klebsiella* spp. *Mycoplasma* spp. (especially M. felis) *Pasteurella* spp. *Peptostreptococcus* spp. *Pseudomonas* spp. *Rhodococcus equi* *Salmonella enterica* serovars *Staphylococcus* spp. *Streptococcus* spp. *Aspergillus* spp. *Mucor* spp. *Cryptococcus neoformans*

1.3.1.1 Viral infections known to cause respiratory disease in horses

Known viruses that affect the respiratory tract of foals include, EIV, EHV-1, EHV-2, EHV-4 and EHV-5, ERV-1, ERV-2, EAdV-1, equine parainfluenza virus 3, and equine reovirus. Equine arteritis (EVA), African horse sickness (AHS) and equine encephalosis (EE) viruses cause systemic disease, usually accompanied by involvement of the respiratory system. The most important viral causes of upper respiratory tract disease in foals are considered to be EIV, EHV-1 and EHV-4 (Mumford *et al*., 1998).

Equine influenza

In areas where EIV is present, it is considered to be the most important cause of acute infectious upper respiratory tract disease in horses (Mumford et al., 1998). This is a highly contagious, acute respiratory disease caused by infection with either A/equine 1 (H7N7) or A/equine 2 (H3N8) influenza viruses.

They are RNA viruses that belong to the family *Orthomyxoviridae* and the genera are classified as A, B and C based upon nucleoprotein (NP) and matrix structural proteins (MP).

They are further grouped into subtypes dependent on neuraminidase (N) and haemagglutinating (H) membrane glycoproteins. Influenza viruses are able to evade pre-existing immunity by undergoing antigenic shift, which is a change in subtype or antigenic drift that are point mutations in the H or N proteins, resulting in gradual changes. Thus they are not only characterized by their surface antigens but also by the geographical origin and year of isolation. Although the equine influenza viruses are antigenically more stable than the influenza viruses of humans, there is some genetic recombination between H7N7 and H3N8 subtypes (Lindstrom *et al*., 1998) and point mutations occur particularly in the H3 haemagglutinin (Hanson *et al*., 1998; Lindstrom *et al*., 1998). Since the recognition of the first type 2 equine influenza virus, known as A/2/Miami/63 (Wadell *et al*., 1963), several lineages have been identified e.g. Kentucky/94 (American-like) and Newmarket/2/93 (Eurasian-like) (Mumford, 1999).

Equine herpesvirus infections

In equids, eight equine herpesviruses have been classified, five in horses and three in donkeys. Equid herpesvirus 1, EHV-3, EHV-4, asinine herpesvirus 1 (AHV-1) and asinine herpesvirus 3 (AHV-3) are members of the Varicellovirus genus of the subfamily *Alphaherpesvirinae* that are rapidly growing cytolytic viruses (Binns *et al*., 1994; Roizman, 1996). Equid herpesvirus 2, EHV-5 and asinine herpesvirus 2 (AHV-2) belong to the slow-growing subfamily *Gammaherpesvirinae* that are associated with B lymphocytes (Agius *et al*., 1994; Browning & Studdert, 1987; Studdert, 1996; Telford *et al*., 1993). These viruses are further characterized by genotyping. An example is EHV-1 that has been further subdivided by gel electrophoresis into 1P (Kentucky D) and 1B (Allen *et al*., 1983).

Of the *Alphaherpesvirinae*, only EHV-1 and EHV-4 are associated with respiratory disease in horses. In addition, EHV-1 is an important cause of abortion, perinatal foal mortality and neurological disease in horses (Martens & Martens, 1991). Infection with EHV-4 manifests primarily as an upper respiratory tract disease, with abortion reported to occur in less than 1 % of herpes viral abortions and only one case of neurological disease being reported (Allen & Bryans, 1986; Meyer *et al*., 1987; Ostlund, 1993). Equid herpesvirus 3 causes self-limiting vesicular lesions on the external genitalia of mares and stallions, the disease being known as coital exanthema.

Equid herpesvirus 2 and EHV-5 usually occur in the upper respiratory tract or in the peripheral blood mononuclear cells of horses (Agius *et al*., 1994). They are often isolated from the upper respiratory tract of healthy horses (Mumford & Rossdale, 1980), and may cause immunosuppression in foals, pyrexia, upper respiratory tract disease, keratoconjunctivitis, malaise and poor performance (Borchers *et al*., 1997; Collinson *et al*., 1994; Dunowska *et al*., 1998; Gibson *et al*., 1992; Kershaw *et al*., 2001; Pälfi *et al*., 1978;

Thein & Böhm, 1976). Equid herpesvirus 2 also predisposes foals to infection by *Rhodococcus equi* (Nordengrahn *et al*., 1996).

Gazelle herpesvirus 1 that was isolated from an outbreak of encephalitis in Thomson's gazelles (*Gazella thomsoni*) in a zoological collection has now been designated equid herpesvirus 9. This is based on its serological cross-reactivity with EHV-1 and EHV-4 and genetic relatedness of the glycoproteins B and G (Taniguchi *et al*., 2000). Experimentally it has caused an interstitial pneumonia and non-suppurative meningitis in horses, goats and laboratory rodents (Taniguchi *et al*., 2000).

Equine adenovirus infections

This virus is a non-enveloped DNA virus belonging to the family *Adenoviridae*, genus Mastadenovirus, that was first isolated from a foal with respiratory disease in the USA in 1969 (Todd, 1969). It is highly stable and thus resistant to changes in pH and temperature. Equine adenovirus has been divided into two serotypes: serotype 1 that is found in the respiratory tract, and serotype 2 that is present in diarrhoeic faeces and lymph nodes (Higashi & Harasawa, 1989). Serological studies (Studdert *et al*., 1974) and DNA restriction analysis (Higashi & Harasawa, 1989) of serotype 1 isolated from various places in the world have found it to be antigenically stable. In healthy foals, EAdV-1 may cause either no overt disease or a mild respiratory disease and diarrhoea (Wilks & Studdert, 1972). However, fatal adenovirus infections occur in Arabian foals suffering from combined immunodeficiency disease (Studdert *et al*., 1974). Adenovirus infections, together with other viruses and bacteria, may cause a mixed infection in foals resulting in an outbreak of upper respiratory disease.

Equine rhinovirus infections

These viruses belong to the family *Picornaviridae*, genus Aphthovirus (Li *et al*., 1997). They are small, non-enveloped double-stranded RNA viruses with cubical symmetry, are resistant to the action of ether and chloroform and are sensitive to pH values of 3 and below. Four viruses have been identified in the respiratory tract of horses, namely, ERV-1, ERV-2, equine rhinovirus 3 (ERV-3) and an acid-stable picornavirus (ASPV) (Thorsen, 1991). The vast majority of rhinovirus infections manifest as subclinical disease. Equine rhinovirus 1 may cause acute pharyngitis in six to nine month-old foals and mature horses (Steck *et al*., 1978, Li *et al*., 1997). Equine rhinovirus 2 is rarely associated with respiratory disease, except in young animals where the pathogenesis is often compounded by secondary bacterial infections. It is believed, however, that rhinoviruses in association with other factors such as mixed viral or bacterial infections, surgery, or strenuous training may cause clinical disease (Thorsen, 1991). Although the other rhinoviruses may cause subclinical infections, they are unlikely to be the cause of overt respiratory disease (Mumford, 1994a; Studdert & Gleeson, 1978).

Reovirus infections

Reoviruses isolated from horses belong to the family *Reoviridae*, genus Orthoreovirus. They are non-enveloped viruses containing segmented, double-stranded RNA that are resistant to the action of ether and chloroform. Reovirus serotypes 1, 2 and 3 have rarely been implicated in clinical respiratory disease, but have experimentally caused a mild upper respiratory disease in 90 and 150 day-old foals (Conner *et al*., 1985).

Parainfluenza virus 3 infections

Parainfluenza virus 3 belongs to the family *Paramyxoviridae*, genus Paramyxovirus. These are pleomorphic, enveloped negative-stranded RNA viruses that are sensitive to lipid solvents, non-ionic detergents, formaldehyde and oxidizing agents. They were isolated in 1961, 1965 and 1969, from 26 of 129 sera (20 %) from horses examined in Philadelphia were found to be positive in a haemagglutination inhibition test (Ditchfield, 1969), which was in contrast to the findings of Todd (1969) who used a haemadsorption neutralization test, similar to that used for bovine parainfluenza viruses, and found no antibodies in 330 sera tested. As the virus has not subsequently been isolated from horses, its significance in upper respiratory disease is unclear.

1.3.1.2 Systemic viral diseases with respiratory manifestations

Equine viral arteritis

Equine arteritis virus (EAV) is a small, enveloped, positive-stranded RNA virus belonging to the family *Arteriviridae*, genus Arterivirus that may cause either subclinical disease or outbreaks of abortion, interstitial pneumonia in young foals and a systemic influenza-like illness in adult horses (Cavanagh, 1997). Although only one serotype of EAV is recognised there are differences in pathogenicity and antigenicity between virus isolates (Glaser *et al*., 1997).

African horse sickness

African horse sickness virus belongs within the genus Orbivirus, family *Reoviridae* and causes a highly fatal disease of horses known as African horse sickness. To date nine serotypes have been identified, all of which occur in South Africa (Coetzer & Erasmus, 1994a). Biting midges of the genus *Culicoides,* and especially *Culicoides imicola* and *C. bolitinos*, are vectors of AHSV (Meiswinkel *et al*., 1994; Meiswinkel & Paweska, 2003). African horse sickness presents itself as one of four syndromes: the pulmonary or peracute form; the cardiac or subacute form; the mixed or acute form that presents signs of both the pulmonary and cardiac forms; and the horse sickness fever form. Clinical signs that would associate it with respiratory disease complex include pyrexia, congested nasal and oral mucous membranes, hyperpnoea and moist rales due to lung oedema.

Equine encephalosis

Equine encephalosis is an orbivirus that is closely related to AHSV and is known to cause a systemic disease in horses in southern Africa. Seven serotypes have been identified, designated 1 to 7 (Howell *et al*., 2002). The morbidity rate may be as high as 60 - 70 % but the mortality rate is less than 5 % (Coetzer & Erasmus, 1994b). About 90 % of affected animals show either no or only mild clinical signs. Initially affected horses have elevated body temperatures, are listless, become depressed and may develop a mild icterus. Less common syndromes that have been attributed to EEV infections include an AHS-like syndrome and abortions (Coetzer & Erasmus, 1994b). Although the vectors for EEV have not yet been conclusively identified, there is sufficient evidence to believe that biting midges of the genus *Culicoides* are involved. It was found that the Bryanston serotype can infect and replicate in *Culicoides imicola* (Venter *et al*., 1999).

1.3.1.3 Bacterial Infections known to cause respiratory infections in horses

Neonatal septicaemia

Suckling foals are usually susceptible to neonatal septicaemia from 2 to 30 days after birth (Platt, 1977) and to respiratory infections from one to six months of age (LéGuillette *et al*., 2002). Approximately 1 % of foals will develop neonatal septicaemia with a third of these cases being fatal (Platt, 1977). Common causes of neonatal septicaemia include *Escherichia coli*, *Streptococcus equi* subsp. *zooepidemicus*, *Actinobacillus equuli* and *Salmonella enterica* serovar Typhimurium (Platt, 1977).

Respiratory infections

Bacteria usually play an opportunistic role in equine respiratory infections, and affect the respiratory tract secondary to virus-induced damage, or other stressors, often resulting in more severe clinical signs (Sarasola *et al*., 1992). These infections are usually polymicrobial in nature with the source being the normal nasopharyngeal flora (Bailey & Love, 1991; Smith & Robinson, 1981). Thus it is important to have an understanding of the normal flora in the nasopharynx in both healthy and diseased horses. There have been extensive investigations in the USA, Canada, United Kingdom and Japan (Bailey & Love, 1991) but there are no published reports of similar studies in South Africa.

Although most bacteria colonizing the upper respiratory tract may occasionally cause respiratory infections, *Streptococcus equi* subsp. *equi*, *Streptococcus pneumoniae* capsular type 3 (Meyer *et al*., 1992), *Rhodococcus equi* and *Burkholderia* (*Pseudomonas*) *mallei* are considered to be true pathogens causing disease in susceptible horses.

Throughout the world *Streptococcus equi* subsp. *zooepidemicus* has been shown to be the most common cause of bacterial infections of the respiratory tract, particularly in four to five month-old foals causing up to 90 % of infections on farms with a history of lower respiratory tract disease (Hoffman *et al*., 1999; Lavoie *et al*., 1991). These infections may occur alone or

as a polymicrobial infection often together with *Pasteurella* spp. (Lavoie *et al*., 1991). Other opportunistic bacteria from the normal nasopharyngeal microflora that are known to cause respiratory disease include other beta-haemolytic streptococci, *Streptococcus suis*, *Staphylococcus* spp., *Acinetobacter* spp., *Actinobacillus equuli*, *Actinobacillus suis*-like bacteria and other *Actinobacillus* spp., *Bordetella bronchiseptica*, *Escherichia coli*, *Pasteurella* spp., *Salmonella enterica* serovars, *Klebsiella pneumoniae* and *Pseudomonas* spp. (Bayley *et al*., 1982; Gallahar, 1965; Jang *et al*., 1987; Lavoie *et al*., 1991; Pace *et al*., 1995; Smith & Robinson, 1981; Sweeney *et al*., 1991). *Streptococcus suis* has been isolated together with *Pasteurella caballi* and *S. equi* subsp. *zooepidemicus* from the lungs of a horse that died of pneumonia (Hayakawa *et al*., 1993). In a study conducted in Pennsylvania, *K. pneumoniae* was isolated from 18 % of horses with pneumonia (Sweeney *et al*., 1991). Although uncommon in primary pneumonia in foals, obligate anaerobes, namely, *Clostridium*, *Eubacterium*, *Peptostreptococcus*, *Fusobacterium* and *Bacteroides* spp. can be important causes of chronic lower respiratory tract disease (Hoffman *et al*., 1993b; Sweeney *et al*., 1985). In South Africa the bacteria most frequently isolated from foals suffering from respiratory tract disease are *S. equi* subsp. *equi*, *S. equi* subsp. *zooepidemicus*, *A. equuli*, A. *lignieresii* and *R. equi* (Results of Diagnostic Bacteriology Laboratory, Department of Veterinary Tropical Diseases, 1996 - 2003).

Bacteria that are frequently isolated from the upper respiratory tract, trans-tracheal aspirates (TTA) or broncho-alveolar lavages (BAL) but are usually of questionable pathogenicity, as they are also isolated from the respiratory tract of healthy horses, include alpha-haemolytic streptococci, (excluding *S. pneumoniae*), *Nocardia* spp. and *Pseudomonas* spp. (Sweeney *et al*., 1989; Wood *et al*., 1993). *Enterobacter agglomerans* and *Bacillus* spp. that are associated with water and soil are often found as transient bacteria on the nasal and bronchial mucosa (Hoquet *et al*., 1985; Sweeney *et al*., 1989).

Although *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* (Lancefield group C) are genetically and phenotypically similar, they differ markedly in biological behaviour. *Streptococcus equi* subsp. *equi* is highly contagious causing the upper respiratory disease known as strangles in susceptible horses, whereas *S. equi* subsp. *zooepidemicus* lives as a commensal on the mucosa of the upper respiratory and external genital tracts of horses causing occasional infections of these areas (Kamada & Akiyama, 1975). *Streptococcus equi* subsp. *zooepidemicus* has, however, been isolated from the lower respiratory tract of horses with respiratory disease in the absence of evidence of a viral infection, suggesting that this bacterium is a primary pathogen (Varma *et al*., 1984).

Isolates of S. equi subsp. equi are usually monogenic, whereas strains of *S. equi* subsp. *zooepidemicus* exhibit great antigenic heterogenecity (Galán & Timoney, 1988). This could be one reason why *S. equi* subsp. *zooepidemicus* is able to survive for extended periods in

the tonsils in the face of local mucosal immune responses (Timoney *et al*., 1994) and account for the repeated and prolonged lower respiratory tract infections (Chanter, 1998).

Streptococcus pneumoniae capsule type 3 causes pneumonia in calves, goats, guinea pigs, dogs, rabbits, rats and horses (Burrell *et al*., 1996). It has been shown experimentally to act as a primary respiratory pathogen in non-exercised horses, causing acute upper and lower respiratory tract disease, and a lobar pneumonia (Blunden *et al*., 1994). In one study in a stable where an outbreak of respiratory disease due to *S. pneumoniae* had recently occurred, the organism was most frequently isolated from subclinically affected horses less than three years old (Burrell *et al*., 1986). It was also, in this study, found to be present on the nasal mucous membranes for up to four months.

Rhodococcus equi, a Gram-positive, facultative intracellular coccobacillus, is the cause of "summer pneumonia" which occurs most commonly in foals between two and six months of age when the maternal immunity wanes (Barton & Hughes, 1980). This bacterium has been isolated from BAL fluids taken from horses with inflammatory airway disease in the absence of seroconversion to the viruses that are known to infect the respiratory tract, namely EHV-1, EHV-4, ERV-1 or EIV (Burrell *et al*., 1994; Hoffman *et al*., 1993a).

Salmonella enterica serovars, particularly Typhimurium, and occasionally Ohio, Enteritidis, Newport, Heidelberg and Anatum, generally cause an enteritis or septicaemia in foals (Pace *et al*., 1995). Affected foals may also have pulmonary abscesses and septic arthritis (Morris Animal Foundation, 1978; Pace *et al*., 1995). *Actinobacillus equuli* is usually associated with septicaemia in neonatal foals, but can in older foals cause pneumonia. *Actinobacillus suis*-like bacteria and a beta-haemolytic *Actinobacillus lignieresii* have been isolated from the upper respiratory tract and tracheal washes of healthy and diseased horses (Samitz & Biberstein, 1991). Unlike the situation in other domestic animals, *Pasteurella* spp. rarely cause respiratory disease in horses. *Mannheimia (Pasteurella) haemolytica*, *Pasturella multocida* and the aerogenic *Pasteurella caballi* have been isolated primarily from the lungs of horses suffering from pneumonia (Pavri & Apte, 1967; Saxegaard & Svenkerud, 1974; Schlater, 1989). Bayley *et al*. (1982), reported *Bordetella bronchiseptica* as a cause of bronchopneumonia in stressed horses in an animal hospital. Horses that had undergone halothane anaesthesia of at least 90 minutes duration represented 67 % of these cases.

Mycoplasmas including *M. arginini*, *M. equirhinis*, *M. equigenitalium*, *M. subdolum*, *M. felis* and *M. salivarium* are commonly found on the mucosa of the upper respiratory tract of horses, where they apparently live as commensals. In Hungary, an unidentified mycoplasma was isolated from the lungs of all foals that died of pneumonia, together with either *Streptococcus equisimilis* or *R. equi* (Antal *et al*., 1988). *Mycoplasma felis* has been implicated as a primary pathogen causing pleuropneumonia in adult horses (Hoffman *et al*., 1992; Ogilive *et al*., 1983; Rosendal *et al*., 1986; Wood *et al*., 1997).

1.3.1.4 Fungal infections known to cause respiratory infections in horses

Although horses and in particular stabled horses are routinely exposed to many fungal species that have the potential to cause inflammation of the respiratory tract, only a few are known to cause disease. In fact, in one study in the USA, 13 % of TTA samples taken from healthy horses yielded fungal growth (Sweeney *et al*., 1985). The most common isolates were *Aspergillus*, *Mucor* and *Penicillium* spp.

Aspergillus fumigatus, although a common saprophyte on decaying plant material, rarely causes nasal and guttural pouch infections and pneumonia (Ryan *et al*., 1992; Sweeney & Habecker, 1999). Other fungi that have been implicated in disease of the upper respiratory tract in horses include, *Rhinosporidium seeberi* causing a chronic polypoid rhinitis (Zschokke, 1913), *Cryptococcus neoformans*, *Coccidioides immitis,* and members of the zygomycetes such as *Conidiobolus coronatus* (Korenek *et al*., 1994). *Cryptococcus neoformans*, *Coccidioides immitis* and *Histoplasma capsulatum* rarely cause pneumonic disease both in neonatal foals and in older horses, usually associated with disease in immunocompromised animals (Rezabek *et al*., 1993; Riley *et al*., 1992).

1.3.2 EPIDEMIOLOGY

1.3.2.1 Viral Infections

Serological and virological studies have found that EHV-1, EHV-2 and EHV-4 (Allen & Bryans, 1986; Borchers *et al*., 1997; Doll & Bryans, 1963; Dunowska *et al*., 1998; Erasmus, 1963; Galosi *et al*., 1994; Studdert *et al*., 1970; Werney *et al*., 1998), EAdV (England *et al*., 1978; Kamada *et al*., 1977; Wilks & Studdert, 1972), and ERV-1 and ERV-2 (DeBoer & Osterhaus, 1978; Holmes *et al*., 1978; Jolly *et al*., 1986; Powell *et al*., 1974; Steck *et al*., 1978; Studdert & Gleeson, 1978; Sugiura *et al*., 1988a; Thorsen, 1991; Werney *et al*., 1998; Wilson *et al*, 1964) are present in most horse-rearing countries in the world.

The large-scale national and international movement of horses, which is usually either by air or by road, for showing, racing, breeding and sale, facilitates the spread of infectious agents (Wilson, 1993). Spread of a disease is further facilitated by inadequate quarantine measures and over-reliance on the efficacy of vaccines.

Equine influenzavirus A subtype 2, the major cause of worldwide epidemics, is endemic in the USA, some countries in western Europe, Scandinavia and Morocco (Chambers *et al*., 1994; El Harrak *et al*., 1998). Epidemics of equine influenza usually occur when partially immune carrier horses have been introduced into regions where the virus does not occur (Mumford, 1999; Powell, 1994). Good examples are the 1986 and 2003 outbreaks of equine influenza in South Africa that originated from horses imported from the USA and England respectively (Guthrie, 2004; Rogers, 1988). As a result of subtype variation, outbreaks of EI still occur in endemic areas. For example, two of the A/equine 2 virus lineages have been found to be co-circulating in the USA, the older A/2/Kentucky/87 and the newer

A/2/Kentucky/91 which is related to the British subtype, A/2/Suffork/89 (Chambers *et al*., 1994). In 1989, in the Jilin and Heilongjiang provinces of north-eastern China a new A/equine 2 strain, avian-like A/2/Jilin/89 (H8N3), thought to have originated from ducks, resulted in 81 % morbidity and up to 20 % mortality in some horse herds (Webster & Guo, 1991). This strain then spread to India (Singh, 1997).

The last recorded outbreak of the more stable A/equine 1 virus was in Russia in 1993 in horses originating from China (Túmová, 1980; Yurov *et al*., 1997). Since 1978 this virus has not been isolated elsewhere in the world, although serological evidence for it has been found in Morocco (El Harrak *et al*., 1998).

In the USA, Australia, Europe and Japan, EHV-4 is most commonly associated with outbreaks of upper respiratory disease in horses (Allen *et al*., 1986; Crabb *et al*., 1995; Suguira *et al*., 1987). In central Kentucky, USA, 46 % and 14 % of outbreaks of respiratory disease have been attributed to EHV-4 and EHV-1 infection respectively (Ostlund, 1993). In Japan EHV-1 was the primary cause of herpesvirus respiratory disease in racing horses (Sugiura *et al*., 1988a). Most foals in Sweden, Iceland and the Sudan are infected with EHV-4 from the time maternal immunity had waned (four to six months of age). These foals were regularly boosted by reactivation of or re-infection with EHV-4 (Allen *et al*., 1998), most infections are, however, subclinical. The results of an epidemiological survey of Thoroughbreds in training in Britain indicated that EHV-1 infections, with or without other viruses, are associated with 45 % of outbreaks of respiratory disease (Powell *et al*., 1978).

By the time they are six months old most foals have been exposed to EHV-4, but this is not necessarily so for EHV-1. Gilkerson *et al*. (1994) found that EHV-4 but not EHV-1 was responsible for outbreaks of EHV respiratory disease on Thoroughbred stud farms in New South Wales, Australia. In central Kentucky, USA, serologic surveys showed that approximately 85 % of foals contract primary EHV-4 infections in their first year of life (Bryans & Allen, 1989).

There appears to be a high prevalence of EHV-2 worldwide, whereas EHV-5 has only been detected in Australia, United Kingdom, Germany and New Zealand (Borchers *et al*., 1999; Browning & Studdert, 1987; Dunowska *et al*., 1998). Equid herpesvirus 2 usually infects foals one to four months of age, causing a mild febrile upper respiratory disease with a persistent cough of one to three months duration (Fu *et al*., 1986; Sugiura *et al*., 1983). Foals tend to shed virus in nasal secretions for two to six months post-infection (Fu *et al*., 1986), the longest period recorded being 418 days (Turner *et al*.,1970).

Less than 50 % of foals in Great Britain are seropositive for EAdV-1 in their first year of life (Burrows & Goodridge, 1978; Gleeson *et al*., 1978). Serologic surveys of racehorses in the USA and Japan indicate that 41 % and 44 % of horses, respectively, in training had antibody titres to EAdV (England *et al*., 1978; Kamada, 1978; Sugiura *et al*., 1988a). In a study done

in Australia and the USA on 631 equine sera, a 73,1 % seroprevalence to EAdV-1 was found in horses (Studdert *et al*., 1974).

In studies done in the United Kingdom, USA, Japan and Switzerland it was shown that horses seroconvert to ERV-1 from two to three years of age (Ditchfield, 1969; Mumford, 1994a; Powell *et al*., 1974). Seroconversion in a racing yard occurred rapidly over a period of four to six weeks with mainly young horses seroconverting (Mumford, 1994a; Powell *et al*., 1974). Seroconversion to ERV-2 occurred from four to six months of age and foals may experience 2 infections within the first year of life (Hofner *et al*., 1978; Mumford, 1994a).

Equine viral arteritis is more common in Standardbreds. Seropositive horses have been identified in many regions in the world including, Japan, Australasia, Great Britain, Europe, North Africa and India (Chirnside, 1992). In South Africa, EAV strains have been isolated from warmbloods and Lippazaners but not Thoroughbreds (Guthrie *et al*., 2003).

Transmission of primary respiratory tract viruses is mainly by inhalation of aerosolized virus particles generated by the frequent coughing of infected horses (Allen & Bryans, 1986). Aerosols of influenza virus can be transported in air currents over distances of at least 32 metres (Wilson, 1993). Other means of transmission include contact via nosing of animals and indirect transmission via contaminated fomites such as halters and personnel failing to observe hygienic precautions when handling and transporting horses (Powell, 1994). Equine rhinovirus 2 infection may also be acquired via the milk during suckling (Holmes *et al*., 1978). Equine arteritis virus can be transmitted both aerogenously or venereally by carrier stallions or by frozen semen (Neu *et al*., 1988; Timoney *et al*., 1986). Although unlikely to be a major route of transmission, a stallion experimentally infected with EHV-1 was found to have infective virus particles in his semen 17 - 25 days post-infection (Tearle *et al*., 1996).

Certain viruses have the ability to remain latent in carrier horses for long periods (Clarke, 1987b). Equid herpesvirus 1 can remain latent in carrier horses life-long. Latency has been demonstrated by cortisone re-activation of clinical disease in horses infected three months previously with EHV-1 (Edington *et al*., 1985; Slater *et al*., 1994a) with the virus being isolated from nasal mucus (Slater *et al*., 1994a), the lymphoreticular system (Browning *et al*., 1988; Scott *et al*., 1983; Slater *et al*., 1994a; Welch *et al*., 1992) and trigeminal ganglia (Baxi *et al*., 1995; Slater *et al*., 1994b). Equid herpesvirus 1 is thought to be harboured either within the blood leukocytes (Browning, *et al*., 1988) or trigeminal ganglia (Borchers *et al*., 1977). Stressors such as weaning, castration, relocation and terminal illness also cause recrudescence of infection with resultant spread of virus to susceptible horses (Browning *et al*., 1988). This phenomenon is of importance as it can initiate an outbreak of abortion in a group of mares. As a result of clinical and epidemiological evidence, it is thought that the same is true for EHV-4. Welch *et al*. (1992) were able detect EHV-4 in leukocytes of foals using genetic typing methods. It has also been suggested that EHV-2 that is harboured in the

B-lymphocytes may play a role in the reactivation of latent EHV-1 and EHV-4 infections (Welch *et al*., 1992).

In the case of ERV-2, there is evidence for a urinary carrier status, as some horses have been found to shed virus in the urine for 116 to 147 days, presumably after bladder invasion by the virus (McCollum, 1992).

In adult horses EAdV may be excreted in faeces for up to 69 days post-infection (Burrows & Goodridge, 1978), indicating that adults may act as inapparent carriers (Powell, 1991).

In areas where one of these viral agents are endemic, clinical disease is usually noticed in foals four to six months of age (that have lost their colostral immunity) or in recently introduced susceptible horses, with mild or no clinical disease in the other animals. Epidemics of respiratory viral disease cause a high morbidity but low mortality rates. Foals and stressed animals, however, show more severe clinical signs.

1.3.2.2 Bacterial and fungal infections

Other than *S. equi* subsp*. equi*, *C. immitis* and *H. capsulatum*, most bacteria and fungi that infect the respiratory tract of horses occur throughout the world. *Histoplasma capsulatum* is endemic in parts of North America with sporadic infections occurring sporadically in the rest of the world (Rippon, 1982). *Coccidioides immitis* is endemic in southwestern USA, Mexico and Central and South America (Rippon, 1982).

Although horses of all ages may become infected and show disease due to streptococcal infections, it is most common and severe in young horses, especially foals between three to eight months of age (Timoney, 1993; Timoney, 1994). In studies in the USA the prevalence of lower respiratory tract disease due to bacterial infections peaked when the foals were between 32 to 180 days of age and on certain breeding farms it was as high as 71 % (Cohen, 1994; Hoffman *et al*., 1993a).

Streptococcus equi subsp. *zooepidemicus* has been found throughout the world, whereas *S. equi* subsp. *equi* is endemic in parts of the world such as Australia, Europe, Britain, North America and Japan (Dalglish, 1992). Between 1978 and 1997, *S. equi* subsp. *equi* was not isolated from horses in South Africa but in 1998, the introduction of carrier horses from Australia resulted in an epidemic of the disease (Bacteriology Laboratory results, Department of Veterinary Tropical Diseases, 1998 – 2004 and Golden Vet Laboratory results, Johannesburg, 2000 - 2002). *Streptococcus equi* subsp. *equi* is shed from nasal discharges and pus of affected animals for up to four weeks and contaminates the environment where the bacteria can remain viable for a month or more. Up to 23 % of convalescent horses can harbour *S. equi* subsp. *equi* in either the guttural pouch or nasopharynx for up to 56 months (Chanter, 1998; Newton *et al*., 1998). Targeted antimicrobial treatment of the guttural pouches can help eliminate carriers (Newton *et al*., 1998). Many horses including those with

no evidence of clinical disease can harbour *S. equi* subsp. *zooepidemicus* in the lymph nodes of the head.

In an Australian study, performed on slaughtered horses and ruminants it was found that *R. equi* was a commensal in the intestines of herbivores. Virulent equine strains are, however, only found in the intestines of foals and adult horses (Takai *et al*., 1994) with the largest numbers occurring in foals within the first eight weeks of life (Hughes & Sulaiman, 1987). Once the bacterium is excreted in a horse's faeces, it becomes saprophytic, growing in organically rich soils due to the presence of organic volatile fatty acids, acetate and proprionate (Hughes & Sulaiman, 1987; Takai *et al*., 1994). This results in an environmental build-up of organisms. Thus *R. equi* infections tend to be localized to a farm, with the number of cases increasing on that farm over the years. Respiratory disease is usually initiated by the inhalation of bacteria from aerosols of dusty soil, or from the faeces or nasal secretions of infected foals (Takai *et al*., 1987).

1.3.3 PATHOGENESIS AND PATHOLOGY

1.3.3.1 Viral infections

Susceptible horses most frequently become infected by inhalation of aerosol particles originating from the nasal secretions of infected horses. The region of viral contact with the respiratory epithelium depends on the size of the virus-carrying particles. Small droplets (<10 µm) can reach the alveoli, whereas larger droplets are deposited in the upper respiratory tract (McChesney, 1975). The anatomic site of exposure will determine whether the viral infection starts as an upper or lower respiratory tract infection. Exceptions to this are viruses that preferably replicate at lower temperatures such as some influenza viruses, herpesviruses and rhinoviruses, which will only replicate in the upper respiratory tract. Equine arteritis virus initially enters into the lung alveoli and multiplies in pulmonary macrophages (McCollum *et al*., 1971).

The incubation period for most respiratory viral infections is short, e.g. 1 to 2 days for EIV (Willoughby *et al*., 1992), 1 to 5 days for EHV-1 (Gibson *et al*., 1992), and 2 to 8 days for ERV-1 (Plummer & Kerry, 1962) and EAV (McCollum *et al*., 1971).

Virus particles attach to specific protein receptors on the epithelium of the nasopharynx and tonsils, replicate and cause hyperaemia and focal erosions in the region, whereafter they spread down the respiratory tract causing laryngitis, tracheitis and bronchitis (Sutton *et al*., 1998). Particularly in the case of EIV infections and to some extent EHV-4 but not ERV-2 infections, mucociliary clearance is decreased, secretions accumulate in the airways, and underlying tissues become inflamed (Willoughby *et al*., 1992). These changes can last for up to 1 month after infection (Willoughby *et al*., 1992). Viruses may then spread to the lower respiratory tract. In a study in the United Kingdom, 66 % of EHV-1 infections resulted in lower

respiratory tract inflammation, which manifested 2 to 12 weeks after initial infection (Burrell *et al*., 1985; Mason *et al*., 1990).

In the case of EIV infections, glycoproteins in mucus bind to the virions preventing attachment to respiratory cells. This is overcome if there is sufficient viral neuraminidase to neutralize the mucus glycoprotein. Influenza virus attaches via its haemagglutinin to a receptor in N-acetylneuraminic acid receptors on the surface of epithelial cells, enter and replicate (Wilson, 1993). Within 1 to 3 days this virus has spread throughout the respiratory tract epithelium aided by cilia, causing epithelial and cilia destruction. Large areas of airway epithelium may become denuded, increasing susceptibility to secondary infections and exposing irritant receptors (Wilson, 1993).

In the case of viruses causing a viraemia such as EHV-1 and less commonly EHV-4, viral particles spread from cell to cell in the respiratory epithelium until they reach the vascular endothelium. Thereafter they are able to infect leukocytes and cause a cell-associated viraemia that usually begins 2 to 3 days post-infection and lasts for 4 to 5 days (Edington & Bridges, 1990; Matsumura *et al*., 1992; Tearle *et al*., 1996) but may last for up to 15 days (Slater *et al*., 1994a). The leukocyte-associated virus then spreads to other sites of the body such as the lymph nodes, central nervous system, kidneys, spleen, and genital tracts. It usually targets the endothelium of blood vessels in target organs resulting in thrombo-ischaemic necrosis (Hamir *et al*., 1994; Tearle *et al*., 1996). The lower respiratory tract is infected through both the air-borne and blood-borne routes with secondary spread to other sites (Kydd *et al*., 1994).

Equid herpesvirus 1 has been detected in lymphocytes, monocytes, macrophages and plasma cells in the lymph nodes, especially of the pharynx and thorax, from 12 hours to 9 days after infection (Kydd *et al*., 1994; Matsumura *et al*., 1992). Once the humoral and cellular immune responses develop the virus is cleared from the respiratory tract and trapped in the reticular endothelial system where it stimulates proliferation of B lymphocytes (Kydd *et al*., 1994). Suppression of T cell functions, lasts for about 4 weeks following EHV-1 infection (Hannant *et al*., 1994b). This immunosuppression is associated with mononuclear cells and/or their secretions rather than circulating antigen or immune complexes (Hannant *et al*., 1994b). It does not appear to be related to the transient lymphocytopenia and neutropenia occurring in horses after EHV-1 infection. Virus-induced immunosuppression is thought to affect the capacity of the animal to respond to other infectious agents or vaccines for several weeks post-infection (Edington *et al*., 1986).

By the second day post-infection EAV reaches the bronchial lymph nodes and from there disseminates, with the exception of the brain, to all body tissues and fluids. It has a tropism for endothelial cells causing a panvasculitis (McCollum *et al*., 1971).

1.3.3.2 Bacterial infections

Viral infections of the respiratory tract or chronic inflammation of the airway can predispose horses to secondary bacterial infections caused by, for example, *S. equi* subsp. *zooepidemicus*, *Pasteurella* or *Actinobacillus* spp. (Mumford *et al*., 1998; Studdert, 1971). Viral infections can impair pulmonary defences, such as particulate clearance and pulmonary macrophage function (Murray, 1989). In neonatal foals Gram-negative sepsis may result in acute respiratory distress syndrome as a result of the pathophysiologic effects of endotoxin (Frevert, 1994).

Streptococcus equi subsp. *equi* invades the mucosa of the nasopharynx causing a pharyngitis and rhinitis. It then spreads to the regional lymph nodes with the development of abscesses that may rupture and drain into the pharynx and guttural pouches or onto the skin causing localized infections. In complicated cases, abscessation of many tissues of the body ("bastard strangles") may develop, or the horses may become sensitized to the streptococcal antigens with the development of purpura haemorrhagica. *Streptococcus equi* subsp. *zooepidemicus* infection results in the development of a purulent inflammatory response present in the regional lymph nodes of the respiratory tract with, at times, involvement of the lungs and pleura. Septicaemia may occur in some affected animals (Martens *et al*., 1981). Both streptococcal subspecies express structurally different M-like proteins (so-called "SeM") that are antiphagocytic and are able to stimulate opsonogenic antibodies (Timoney *et al*., 1994) and IgA (Timoney *et al*., 1998). Other virulence factors that are expressed by both subspecies include an antiphagocytic hyaluronic acid capsule, hyaluronidase, streptokinase, cytotoxic streptolysin O, inflammation stimulating cell wall peptidoglycan, and albumin and IgG Fc-receptor cell wall binding proteins (Timoney, 1993).

The mortality rate in foals less than three months of age due to *R. equi* infections is up to 80 %. It causes an acute or chronic suppurative bronchopneumonia, particularly of the cranial and cardiac lobes. Caseo-necrotic foci resembling abscesses are frequently part of the pneumonic process. A regional lymphadenitis of the mesenteric lymph nodes is often present. An extensive pyogranulomatous typhlocolitis with multiple ulceration accompanies the pulmonary lesions in a high proportion of cases. Abscessation of other organs is rare (Leadon, 1989). Many parts or products of these bacteria such as mycolic acid containing glycolipids in the cells wall, capsular polysaccharides, cholesterol oxidase, choline phosphatase and phospholipase C exoenzymes are thought to play a role in virulence. However, only bacteria that contain plasmids which are able to code for 15 or 17-kiloDalton lipoproteins, known as VapA, on their surface causes the typical lesions in foals as well as the development of a protective immunity in these animals (Giguère & Prescott, 1998; Machang'u & Prescott, 1991). This protein is only expressed in large amounts at temperatures between 34°C and 41°C and is thought, together with co-factors VapC, D and

E, to enhance intracellular survival in the macrophages and induce both humoral and cellular immunity (Giguère & Prescott, 1998).

1.3.3.3 Fungal Infections

Mould spores present in rotting plant material such as hay baled with a high moisture content, bedding containing wood shavings and paper used as deep litter, form a major constituent of dust to which horses and in particular stabled horses are exposed (Clarke, 1987a). Furthermore moist environments may promote the growth of fungi. Although inhaled, mould spores rarely invade the respiratory tract to cause, for example, nasal and guttural pouch infections and pneumonia, they are more commonly associated with allergic disease.

Stabled horses are regularly exposed to at least 70 fungal species, all of which have the potential to induce an inflammatory response. Long-term exposure of horses to high numbers of fungal spores can result in chronic pulmonary disease (Clarke, 1987a). The inhalation of fungal spores may also induce covert respiratory disease, in the form of lower respiratory tract inflammation, and lengthen the recovery period of other infectious diseases (Clarke1987b).

1.3.4 DEFENCE OF THE EQUINE RESPIRATORY TRACT TO INFECTIOUS AGENTS

1.3.4.1 Colostral immunity

Although the neonatal foal is able to produce autogenous gammaglobulins from two weeks of life, adequate amounts are not produced until four months of age. No significant transfer of immunoglobulins occurs during gestation, since the placenta of the mare is of the diffuse chorioepithelial type. By providing antibody-containing colostrum, mares are able to give their new-born foals some temporary passive protection against a range of micro-organisms to which they have been previously challenged (Jeffcott, 1974). Failure to receive adequate amounts of colostrum can result in severe infectious disease or even death of neonates from two to 30 days of age (Platt, 1977; Robinson *et al*., 1993).

Foals may fail to receive colostrum-derived immunoglobulins in the following situations:

- Premature lactation, presumably associated with hormonal changes, results in colostrum containing very little antibodies. This is the most important cause of a lack of passive immunity in new-born foals (Rumbaugh *et al*., 1979).
- Primigravous mares or mares older than 15 years of age sometimes fail to produce colostrum containing sufficient immunoglobulins (Le Blanc *et al*., 1992; Rumbaugh *et al*., 1979).
- Foals that have no or delayed access to colostrum as a result of being born weak or deformed (Rumbaugh *et al*., 1979).
- Adrenocorticoids produced by either the mare or foal under stress conditions can lead to a change in the permeability of enterocytes and thus to the absorbency of

macromolecules found in colostrum (Jeffcott, 1972). The permeability of the intestinal mucosa to macromolecules in stressed foals can be reduced to within four to six hours after birth.

- Mares whose foals are born before 320 days of gestation do not produce colostrum due to inadequate hormonal stimulation (Rumbaugh *et al*., 1979).

Colostral antibodies are predominantly IgGb with smaller amounts of IgGa, IgG(T) and IgA (Sheoran *et al*., 2000). The foal's intestinal cells take up these immunoglobulins within 24 hours of birth. Peak serum gammaglobulin levels are attained by 18 hours of life and are usually the same or higher than that of the serum of their dams but lower than the colostral antibodies (Sheoran *et al*., 2000). In milk the major immunoglobulin isotype is IgA that may have a protective effect on the gastrointestinal tract (Sheoran *et al*., 2000). In nasal secretions protective maternally-derived antibodies, usually IgGa and IgGb, are found up to 42 days of age and IgA after 28 days of age (Sheoran *et al*., 2000).

Foals are able to produce IgGa before birth but usually don't produce IgGb until six days after birth with sufficient levels only occurring at 63 days of age (Sheoran *et al*., 2000). Colostrum-derived antibodies begin to wane at two to three months of age, causing foals to become susceptible to a considerable number of infections, both bacterial and viral, particularly of the respiratory tract.

1.3.4.2 Mucosal, humoral and cellular immunity

The probability of an infectious agent inducing respiratory disease in a horse is dependent on the pathogenicity and infectious dose of the pathogen, and the susceptibility of the horse (Clarke, 1987b). Particles deposited in the airways and lungs are removed by the mucociliary escalator and alveolar macrophages respectively (Brain & Valberg, 1979). A local mucosal immune system consisting of mucosal lymphoid tissue, mast cells, macrophages, immunoglobulin-producing plasma cells, and free immunoglobulins deal with infectious agents at their site of deposition (Clarke, 1987b). The local mucosal immunity is considered to be of major importance in the defence against respiratory pathogens such as EIV (Rouse & Ditchfield, 1970), EHV (Breathnach *et al*., 2001) and S. equi subsp. equi (Galán & Timoney, 1988) infections. Disturbances in this immunity such as strenuous exercise may increase the horse's susceptibility to respiratory disease was demonstrated by Folsom *et al*. (2001). Immunity after natural infections or immunization are not always effective or long-lasting. Natural immunity tends to be short-lived in respiratory disease where the infection is localized to the point of entry e.g. upper respiratory tract and where there is a short incubation period (Bryans, 1981), and generally strong and long-lasting where systemic spread of the agent occurs (Clarke, 1987b). Following an episode of respiratory disease associated with EHV-1 or EHV-4, the protective immunity to a subsequent bout of disease caused by the same organism may be as short as two months (Thomson, 1978). However,

the length of resistance to abortion associated with EHV-1, which requires a systemic spread of the virus to reach the foetus, is much longer and may in some horses be life-long (Allen & Bryans, 1986). Natural immunity to ERV is long-lasting and serotype-specific (Ditchfield, 1969).

Both humoral and cellular immunity are required for host defences against a number of respiratory pathogens and are usually highly specific. In horses that have been naturally infected with EIV low levels of serum antibody are protective against rechallenge, affording a protective immunity that can last for a year or even longer in some animals (Hannant *et al*., 1988). This is most probably because natural infection not only stimulates antibodies of the IgG class, but also local mucosal antibodies of the IgA and IgM classes (Hannant *et al*., 1989; Rouse & Ditchfield, 1970) and cell-mediated responses (Hannant *et al*., 1989). In fact, there is a decrease in virus shedding with an increase in mucosal antibodies (Breathnach *et al*., 2001; Hannant *et al*., 1989). Recently cell-mediated responses have been found to play a major role in protective immunity to EIV. Natural infection by EIV stimulates memory T cell proliferative responses that on re-challenge stimulate cytotoxic T lymphocytes to clear virus from the body (Hannant *et al*., 1994a). Horses naturally infected with S. equi subsp. equi have elevated serum protein M antibody (SeM) IgG and nasopharyngeal IgA and IgG antibodies and are highly resistant to re-infection (Timoney *et al*., 1998).

Animals that recover from EAV infection produce high levels of neutralizing antibodies, which correlate well with protection from re-infection of the respiratory tract but not that via the venereal route of infection (McCollum *et al*., 1988).

1.3.5 CLINICAL SIGNS OF RESPIRATORY DISEASE

Upper respiratory tract disease is usually associated with a primary viral infection with secondary bacterial invasion of the damaged respiratory mucosa. Primary bacterial and fungal infections are rare. Most of the viruses cause mild or subclinical infections of the upper respiratory tract with the exception of EIV, EHV-1 and EHV-4 that can cause severe disease especially in susceptible horses (Bürki & Sibalin, 1972; Hofer *et al*., 1973). However severe disease may occur in immune-suppressed, or debilitated horses (Thorsen, 1991; Wilks & Studdert, 1972).

Most of the viruses that affect the upper respiratory tract such as EIV spread rapidly with EHV-1, EHV-4 spreading more slowly. They all, however, within the course of two to three days throughout a susceptible population, causing upper respiratory disease of high morbidity but low mortality (Gibson *et al*., 1992).

One or more of the following clinical signs may occur in an animal suffering from upper respiratory disease (McChesney, 1975): [Note that the clinical signs of upper respiratory disease due to EHV-1 infections, although resembling those of EHV-4, tend to be milder (Gibson *et al*., 1992).]

- Fever (1,5 to 3,5 °C above baseline).
- Mild to severe anorexia, dependent on the severity of the lesions. In EHV-4 infections there can be up to a 50 % reduction in feed intake (Willoughby *et al*., 1992).
- Enlarged, oedematous pharyngeal and mandibular lymph nodes (Gerber, 1969). They do not appear to be painful in the case of EHV-1 infections (Willoughby *et al*., 1992).
- Serous to mucopurulent nasal discharge. Thin watery mucus is suggestive of a viral infection, whereas a thick, green exudate suggests bacterial activity. A slimy clear mucus is common with adenovirus infection (McChesney, 1975).
- Paroxymal, dry cough, which is especially associated with EIV infections (Gerber, 1969) compared to an infrequent, moist cough which may be noted in equid herpesvirus infections (Mumford & Rossdale, 1980)
- Different grades of severity of pharyngitis. Ulcerative lesions in the pharyngeal region have been associated with EHV-4 infections (Studdert *et al*., 1970).
- Conjunctivitis, epiphora, limb oedema, muscle stiffness, tachypnoea or dyspnoea and tachycardia are often associated with EIV infections (Timoney, 1996).

Clinical signs associated with lower respiratory disease that correlate well with endoscopic, haematological and bronchial lavage cytological findings are (Hoffman *et al*., 1993a):

Abnormal lungs sounds on auscultation, including wheezes, crackles and/or loud bronchial tones elicited after re-breathing into a three litre plastic bag.

- Pyrexia (39 - 42°C).
- Cough, usually soft and moist, especially on exertion (Bürki & Sibalin, 1972).
- Bilateral mucopurulent nasal discharge or crusting around the nostrils or on the dorsum of the canon bones.
- Tachypnoea (>40 inspirations/minute).
- Lethagy.

Decreased lung sounds and dull sounds on percussion may be indicative of abscesses or pleural effusion. In severe cases, abdominal breathing, flaring of the nostril, cyanosis and pronounced exercise intolerance are present (LéGuillette *et al*., 2002).

Unless complications occur most horses recover from EHV-1 infections by day nine after infection (Gibson *et al*., 1992). Complications of viral infections include secondary bacterial infections, chronic bronchiolitis that may lead to chronic obstructive pulmonary disease, guttural pouch infections, sinusitis, myocarditis, myositis and liver damage. A rare

complication is laryngeal hemiplegia and pharyngitis. Tracheal clearance in EHV-1 and EHV-4 infections are markedly impaired for up to 30 days post-infection (Willoughby *et al*., 1992).

Secondary bacterial infections tend to cause a fever of higher and longer duration than the first, two to three days after the initial viral-induced pyrexia. This is associated with a mucoid to mucopurulent nasal discharge, exacerbated coughing and signs of bronchiolar and lung involvement. If left untreated, bacterial infections can result in bronchopneumonia and pleurisy (Timoney, 1996).

1.3.6 CLINICAL PATHOLOGY FINDINGS

Changes in haematological and blood chemistry parameters, following infection may vary, dependent on the severity and stage of disease as well as the age and fitness of the horse, making it difficult to interpret the results. Serial daily sampling of blood from horses experimentally infected with EHV-1 or EHV-4 showed a sudden decrease in circulating blood lymphocytes and neutrophils, accompanied by monocytosis. This represents the earliest haematological indication that a virus infection is present (Mason *et al*, 1990; Mumford & Rossdale, 1980). In primary infections with EHV-1 there is a biphasic lymphopenia and neutropenia on day two and days six to seven after infection (Gibson *et al*., 1992) In bacterial pneumonia a leukocytosis and hyperfibrinogenaemia (> 8g/L) may be present (LéGuillette *et al*., 2002).

1.3.7 DIAGNOSIS OF VIRAL, BACTERIAL AND FUNGAL INFECTIONS OF THE RESPIRATORY TRACT

The diagnosis of a specific aetiological agent as a cause of respiratory disease can be difficult as the clinician is usually only consulted after the disease has been present for a while with the result that secondary bacterial infections have occurred. At this time it may not be possible to identify the primary aetiological agent in all clinical cases.

The diagnosis of infectious respiratory disease is based on four principals:

- Recognition of clinical disease
- Ancillary procedures
- Culture or detection of the causative organism/s
- Serology

1.3.7.1 Recognition of clinical disease

The following clinical signs may be noted: fever, anorexia, cough, nasal discharge, ocular discharge, congestion of the nasal mucosa, enlarged pharyngeal lymph nodes, enlarged guttural pouches, dyspnoea, lung consolidation on percussion and abnormal respiratory sounds on auscultation.

1.3.7.2 Ancillary procedures

Various ancillary procedures are used to assist in the diagnosis. These include direct visualization of affected regions by laryngoscopy or bronchoscopy and indirectly by radiography or ultrasonography. Material obtained from affected areas, either by TTA, BAL or fine needle aspiration of lung tissue, is used in the study of the cytology and for detection of the causative agent. Samples of blood are used to measure fibrinogen levels, and complete blood cell counts and gas analyses of arterial blood aid in the prognosis and therapy of affected horses (Hondalus & Paradis, 1989)

1.3.7.3 Specimen collection for the isolation/detection of the causative agent/s

The nature of samples that are taken from live animals are dependent on the pathogen or pathogens suspected and the location of the disease process. They may consist of heparinized blood, clotted blood for serology, nasopharyngeal swabs or washings, TTAs, BALs, lung aspirates and pleural fluid (Fukunaga *et al*., 1981; Hoffman & Viel, 1997; Hondalus & Paradis, 1989).

Viruses causing disease of the upper respiratory tract are usually detected by the use of deep nasopharyngeal swabs (Hoffman & Viel, 1997). If a viraemia is present, e.g. in EHV-1 and EAV, virus can be isolated from a buffy coat blood sample. Equine adenovirus can also be isolated from conjunctival mucosa scrapings and ERV-2 from the urine (McCollum, 1992). Since viruses causing respiratory disease are only present in the nasopharynx and blood for a short period, blood from which serum is separated for serological tests is collected from horses in the acute and convalescent phases of the disease. Samples for virological recovery and serology are usually submitted to the laboratory at 4°C.

Nasopharyngeal swabs are rarely used for the diagnosis of bacterial infections, as most of the bacteria that are opportunistic pathogens are also normal commensals of the upper respiratory tract mucosa. Thus for lower respiratory tract infections a TTA, BAL, pleural fluid aspirate or lung biopsy are preferred. Since fungi may affect the upper respiratory tract causing guttural pouch and nasal mycoses, fluid flushes can be made of these areas and submitted for culture. The confirmation of infection is, however, usually by the demonstration of fungal hyphal invasion of tissue, either on cytology or histopathological examination.

Samples for the aerobic isolation of bacteria and fungi can be simply preserved in a non-nutritive, osmotically balanced, moist medium such as phosphate buffered saline (PBS), while samples taken for anaerobic isolation require a pre-reduced transport medium.

1.3.7.4 Isolation or detection of the causative virus

An aetiologic diagnosis can either be made by isolation and identification of the agent, or detection of antigen by means of molecular or immunological techniques.

Culture and identification:

Although the culture and subsequent identification of viruses is relatively slow and labour intensive, it is usually indicated when the causative agent is unknown or when the detection of viable viruses is essential. Isolation of virus from the upper respiratory tract may not in all cases be possible, as excretion of virus usually coincides with pyrexia, with the more obvious signs of respiratory disease only occurring after the excretion of virus has ceased. For example, horses suffering from EHV-1 induced disease for the first time may excrete virus for a week or more. In subsequent infections, this period lasts for only 24 to 48 hours with low virus concentrations in exudates (Mumford & Rossdale, 1980). Equine influenza virus is usually only isolated from deep nasal swabs for two to four days post-infection (Evermann *et al*., 1987; Wilson, 1993).

With the exception of EIV, most known respiratory viruses are cultured in tissue cultures of Vero (established cell line from African green monkeys), primary foetal equine lung cells and rabbit kidney cells (RK13). Equid herpesvirus 1 but not EHV-4 replicate in RK13 cells, incubated at 37 °C. Equid herpesvirus 2 will replicate well in RK13 tissue cultures, taking between 6 and 28 days to be detectable. Equine rhinovirus and EAdV can be cultured in RK13 cells, equine foetal lung and equine foetal kidney (Studdert *et al*., 2003). Equine arteritis virus is cultured on Vero tissue cell cultures with the cytopathic effect (CPE) being only detected after 2 to 8 passages (Golnik *et al*., 1981). Equine influenza virus can be isolated by first treating centrifuged specimens with neuraminidase and then inoculating them into 10-day-old embryonating hen's eggs or Madin-Darby canine kidney cells (MDCK). The presence of EIV in cells is detected by haemagglutination tests (Hoffman *et al*., 1999).

Molecular and immunological methods

Molecular and immunological techniques have recently been developed that are in many instances more sensitive and specific, and faster to perform than the traditional methods used in diagnostic laboratories.

Immunological techniques that are used include immunohistochemical techniques, capture ELISA or immunofluorescent tests (IFAT). Immunofluorescent techniques identify viral inclusion bodies in nasal or conjunctival mucosa cells. Early in the course of equine influenza the virus can be identified in cells obtained from nasopharyngeal swabs using a direct IFAT based upon the detection of influenza A viral nucleoprotein (Ånestad & Maagaard, 1990; Timoney, 1996), or an antigen capture ELISA incorporating monoclonal antibodies to A/equine/H3N8 (Cook *et al*., 1988; Livesay *et al*., 1993). The indirect IFAT is considered to be more sensitive and faster than viral isolation for the identification of EHV-2 isolates (Mumford & Thomson, 1978b)

Polymerase chain reaction (PCR) assays of viral DNA or RNA has been developed for most of the viruses that infect the respiratory tract, including EHV-1 and EHV-4 (Binns *et al*., 1994;

Lawrence *et al*., 1994; Matsumura *et al*., 1994), EHV-2 and EHV-5 (Agius *et al*., 1994), and EIV (Oxburgh, 1999). A nested PCR assay has been used to identify EAV in semen (Gilbert *et al*., 1997), blood and nasal secretions (McCollum *et al*., 1999).

1.3.7.5 Serology

Serological techniques as they detect the humeral immune response to a disease, are used in the retrospective diagnosis of respiratory infections as well as in epidemiological surveys of the presence or absence of a disease. Serological results should be interpreted with care as antibody levels can remain elevated long after the virus has been cleared from the horse. They may also be elevated as a response to administration of vaccine or, in foals, to the transfer of maternal antibodies. A rise in specific IgM or a four-fold rise in specific IgG over a 10 to 14 day period is generally considered to be indicative of a recent infection. Serological tests for the diagnosis of viral agents are selected on the basis of specificity, sensitivity and the ease with which the technique can be performed.

For the diagnosis of EIV and equine reoviruses the easiest tests to use are the indirect haemagglutination test that detects both IgM and IgG immunoglobulin subtypes or preferably the radial haemolysis test (Conner *et al*., 1985; Frerichs, *et al*., 1973; Plateau & Cruciere, 1983, Rogers, 1988; Schild *et al*., 1975; Schmidt, 1976).

Several tests have been used to detect antibodies to equid herpesviruses (Mumford & Thomson, 1978a). The most specific but technically difficult test is the serum neutralization test (SNT) (Burleson *et al*., 1992). This test is predominantly type specific for EHV-1 but horses infected with EHV-4 will develop cross-reactive antibodies to EHV-1. The complement fixation test (CFT) has been most widely used for the diagnosis of the equid herpesviruses (Fu *et al*., 1986; Sugiura *et al*., 1988b). In the case of EHV-1 and EHV-4 complement fixing antibodies develop ten days earlier and last for ten weeks shorter than neutralizing antibodies (Suguira *et al*., 1988b; Thomson *et al*., 1976). The concentration of antibody in the serum gradually increase over time in foals persistently infected with EHV-2 reaching their peak at the time when virus can no longer be cultivated, and thereafter they slowly decrease (Fu *et al*., 1986). Recently a highly sensitive type-specific ELISA has been developed using as antigen glycoprotein G (gG) from either EHV-1 or EHV-4 homologues expressed in *E. coli* (Crabb *et al*., 1995).

For the diagnosis of equine rhinovirus and EAV infections, the SNT is used (McCollum & Bryans, 1972). Serological tests that are suitable for demonstration of antibodies to EAdV include the HI and SNT (Kamada, 1978).

For the diagnosis of EAV infections, the SNT can be used (Senne *et al*., 1985).

1.3.7.6 Bacterial and fungal infections

Identification of bacteria (with the exception of mycoplasmas) and fungi usually presents no problems as they grow on standard bacterial growth media and can be identified by biochemical tests (Carter & Cole, 1990; Holt & Krieg, 1984; Holt *et al*., 1986, 1989a, 1989b, 1989c, 1994; Koneman *et al*., 1995; Lennette *et al*., 1985; Quinn *et al*., 1994). All aerobic and facultative aerobic bacteria that infect the respiratory tract of horses can be grown on Columbia blood agar and incubated in a candle jar containing approximately 3 % carbon dioxide at 37 °C. Most bacteria will grow within 18 to 24 hours.

Streptococcus equi subsp. *equi* and *S. equi* subsp. *zooepidemicus* tend to form mucoid colonies with a wide zone of beta-haemolysis on blood agar. These Gram-positive cocci are further identified by being typed as Lancefield group C. *Streptococcus equi* subsp. *equi* does not ferment lactose, trehalose and sorbitol whereas *S. equi* subsp. *zooepidemicus* ferments sorbitol and lactose (Carter & Cole, 1990).

Streptococcus pneumoniae is a fastidious organism and will only grow under microaerophilic conditions in media enriched with purines and pyrimidines. Identification is based upon the presence of alpha-haemolytic, mucoid, transparent colonies consisting of Gram-positive cocci that are catalase-negative and sensitive to optochin (Quinn *et al*., 1994).

Rhodococcus equi can easily be isolated in aerobic conditions on standard laboratory media on which, after 48 hours incubation, it forms pink/red beta-haemolytic, mucoid colonies. Identification is then based upon enhancement of haemolysis using either Staphylococcus aureus or Listeria monocytogenes beta-haemolysins. The organism does not ferment sugars and is catalase and oxidase negative (Carter & Cole, 1990).

Members of the Enterobacteriaceae grow well under aerobic conditions on blood tryptose agar as well as on MacConkey agar (Quinn *et al*., 1994). On MacConkey agar, *E. coli* colonies are pink, *K. pneumoniae* colonies are large pink and mucoid and *Salmonella* colonies are spreading and colourless. *Salmonella* serovars produce hydrogen sulphide that causes colonies on XLD agar and triple sugar iron agar slants to be black. A panel of biochemical tests and a triple sugar iron slant are employed for the identification of members of this family (Lennette *et al*., 1985). *Klebsiella* isolates can be further characterized by capsular serotyping, and E. coli and Salmonella serovars by somatic (O) and flagellar (H) antigen typing (Lennette *et al*., 1985).

Actinobacillus spp. form grey translucent colonies on blood agar after 24 hours of incubation at 37 °C. Identification of *Actinobacillus* spp. is based on their ability to produce urease and upon conventional biochemical tests to confirm their identity (Carter & Cole, 1990; Phillips, 1984).

Pasteurella spp. form small translucent to grey colonies of blood agar and have a characteristic smell resembling that of rodent urine. Identification of *Pasteurella* spp. is done by growth on MacConkey agar and positive oxidase, gelatinase, urease and nitrate reduction tests as well as fermentation profiles of a variety of sugars (Mannheim, 1984).

Since mycoplasmas are sensitive to environmental conditions, especially desiccation, they should preferably be plated immediately, after collection of the samples, onto an artificial growth medium. As they lack a bacterial cell wall they have specific growth requirements, and require a medium containing serum and cholesterol such as Chalquest and Hayflick's medium for growth (Hayflick, 1965). Most mycoplasmas grow better if incubated in an atmosphere of 5 % carbon dioxide and high humidity at 37 °C. Identification is based upon growth inhibition or direct fluorescent antibody tests using species-specific antisera (Oligivie *et al*., 1983).

Most fungi, including saprophytes, can be isolated on Sabouraud's dextrose agar and grown at 25 °C. Fungi that are animal host-adapted will also grow on brain heart infusion agar at 37 °C. Identification is based upon colony and microscopic morphology as well as biochemical tests (Larone, 1995). Further characterization of animal pathogens such as *C. neoformans* can be done using immunological tests (Campbell & Stewart, 1981).

1.3.8 TREATMENT AND CONTROL

Since there are no antiviral agents registered for the specific treatment of horses, treatment of virus-induced disease involves symptomatic therapy, provision of rest and, if indicated, antimicrobials to prevent secondary bacterial infections. The specific treatment of bacterial infections is best done by the selection of antimicrobials based upon the results of an antimicrobial sensitivity test and knowledge of the pharmacokinetics and pharmacodynamics of the selected antimicrobial in the horse.

The control of respiratory infections is determined by its perceived impact on the animal population, how contagious it is and what the disease consequences are to the animal/s should they become infected.

Several methods are used to prevent disease or its spread and include methods aimed at decreasing the susceptibility of the animals and decreasing the concentrations of the infectious agent. These include immunoprophylaxis, environmental sanitation, the segregation of susceptible animals from potential carriers, the treatment of sick animals and, where applicable, vector control. To select the correct methods, the epidemiology and pathogenesis of each infectious agent should be fully understood. In highly contagious diseases such as equine influenza, it is best to routinely immunize using the appropriate influenza virus strain and, should an outbreak occur, to quarantine the entire premises until the horses are no longer infective. In the case of equid herpesviruses that require close contact for spread to occur it may be sufficient to routinely immunize and physically separate

clinically affected horses or those who have recently aborted from healthy horses. Bacterial infections, such as R. equi that originates from the faeces of foals and is an environmental saprophyte, are controlled by regular removal of faeces, rotation of foaling camps and the administration of hyperimmune plasma to neonatal foals at risk.

Good neonatal management is beneficial in protecting foals against most infectious diseases. This involves foaling down in a clean environment, routine disinfection of the umbilicus, keeping foaling groups separate from other horses on the farm and, most importantly, ensuring the adequate intake of good quality colostrum within six hours of birth. Should the latter not be possible, fresh plasma from an older, healthy mare can be administered intravenously in the selected foal.

CHAPTER TWO : MATERIALS AND METHODS

2.1 STUDY GROUPS AND SAMPLING

2.1.1 EXPERIMENTAL ANIMAL POPULATION

Thirty Thoroughbred foals, bred on Bosworth breeding farm, situated 15 km North of Klerksdorp, North-West Province, South Africa (Figure 1), were monitored for infectious respiratory disease from birth until one year of age. Reasons for selection of this farm and these horses were as follows:

- Closest large Thoroughbred stud farm to the Faculty of Veterinary Science, Onderstepoort, facilitating regular collection of samples and collaboration with the owners of the stud.
- The stud was very well managed and thus good records were kept.
- On average 60 foals were born annually on the farm, allowing sufficient numbers to be selected.
- The animal population on the farm was relatively stable, thus enabling a study over a one-year period.
- The farm had a history of respiratory disease in the foals during the post-weaning period.

2.1.1.1 Farm management and background

Housing:

At the time of the study Bosworth farm housed approximately 80 mares, 60 foals and 4 stallions, 2 of which were teaser stallions. Adult horses were usually kept in camps (Figures 2 & 3), with barren mares, pregnant mares and stallions being separated. Mares were individually stabled overnight and were kept in small groups, in paddocks during the day for one week prior to their expected foaling date. After partus they were kept together with their foals in individual camps for approximately one week, but the duration of this period varied dependent on the health status of the foal and mare. Thereafter suckling foals were kept with their dams in larger camps in groups of similar age and the same sex. At 6 months of age, they were weaned as a group and placed in camps on the other side of the farm to the mares (Figure 4). Fillies and colts were maintained in separate camps. More valuable foals were stabled at night. A month before the yearlings were sold, they were stabled at night and brought into condition for sale.

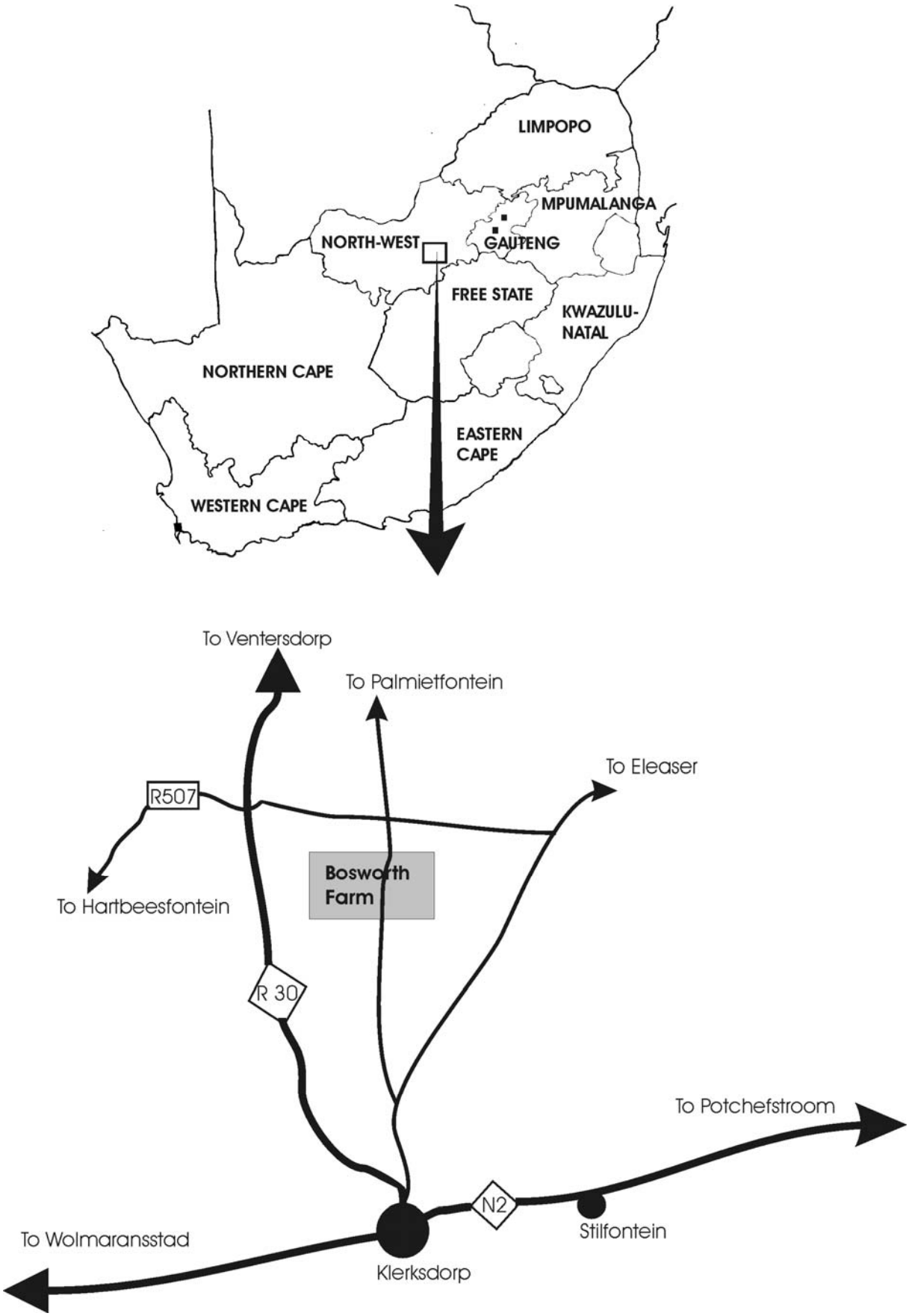

Figure 1: Geographical position of Bosworth farm in South Africa.

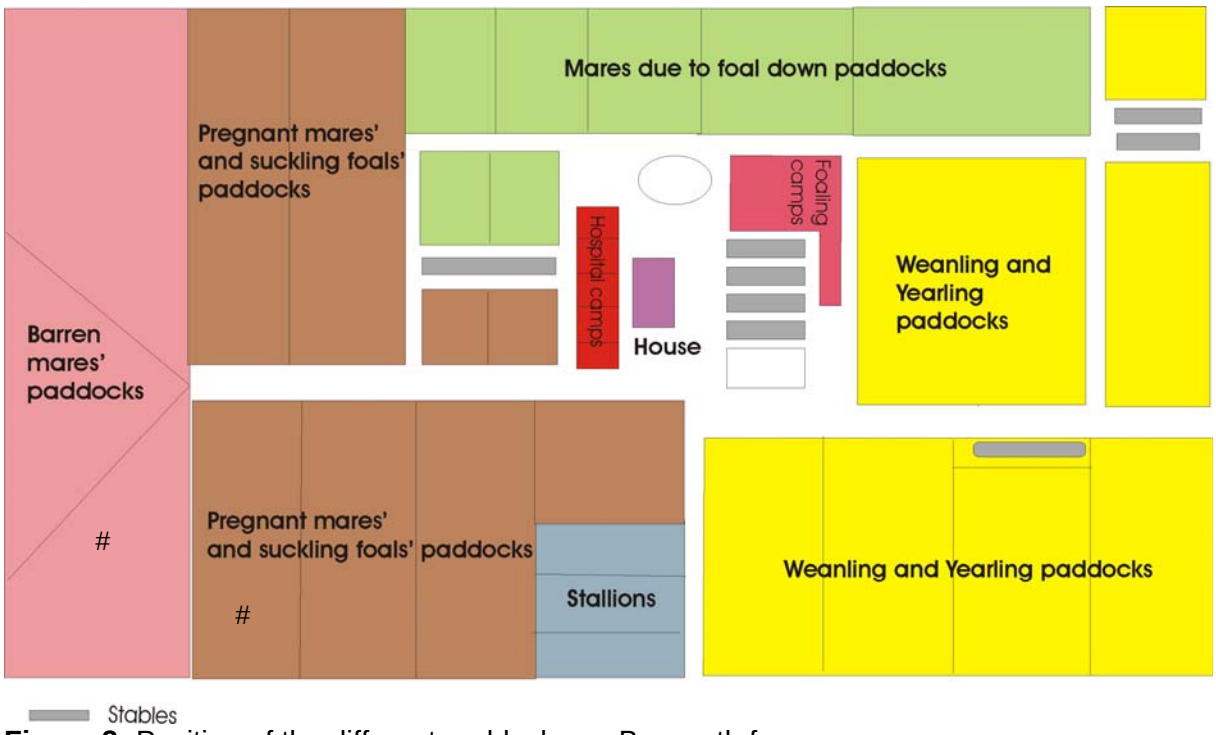

Figure 2: Position of the different paddocks on Bosworth farm.

Figure 3: A paddock in which mares and foals at foot are kept. Photographed in January 1995. (This is one of the paddocks indicated in brown in Figure 2).

Figure 4: Paddocks in which the weanlings and yearlings were kept in winter. Photographed in June 1995. (These paddocks are indicated in yellow in Figure 2).

2.1.1.2 Nutrition

Although natural grazing was available (so called "sourveld") its quality varied with the season and rainfall. The lactating mares are given 8 kg of a mare mix consisting of molasses meal, yellow maize meal, wheat bran, rolled oats, lucerne meal and Epol FARMIX 350^{TM}, a protein and vitamin supplement. In each camp teff hay (*Eragrostis tef*) of high quality was provided *ad libertum* in a large covered crib. Suckling foals also had access to this feed prior to weaning. Large water troughs served two adjacent camps. After weaning, the foals were provided with teff and rye grass (*Loluim* sp.), hay *ad libertum*, and each also received 6 kg per day of a similar concentrate ration as was given to the mares except that the yellow maize meal was replaced by additional lucerne.

2.1.1.3 Environmental conditions

The farm was situated in a summer rainfall area with an average annual rainfall of 796 mm with most of the rain falling between October and March. The winter night temperatures average 9 °C and the daytime average was 17 °C, and in the summer the average was 12 °C at night and 26 °C during the day. During the summer temperatures reached 36 °C on some days.

A stream, indicated on Figure 2 by the icon #, runs through the farm, causing two of the low-lying camps to be flooded in summers of high rainfall as was the case during the period of this study. “Summer itch” a hypersensitivity reaction of horses to the bite of *Culicoides* spp. is

more common in these camps. Older vaccinated horses are usually housed here as the likelihood of contracting AHS is greater in the low-lying areas.

2.1.1.4 Veterinary interventions

At 6 months of age the foals were vaccinated against tetanus using “Tetanus OBP” (Onderstepoort Biological Products, South Africa), and EIV and EHV-1 and EHV-4 using "Fluvac EHV 4/1 Plus” (Fort Dodge, USA). A booster vaccine was administered one month later. Thereafter all horses on the farm were vaccinated annually. Every year, in September, all horses except for those less than 6 months old were vaccinated against AHS using the live attenuated polyvalent “Horsesickness OBP” (Onderstepoort Biological Products, South Africa) vaccine. Because 2 mares aborted and 4 mares had neurological signs caused by EHV-1 infection in late July and early August 1993, all mares in foal were vaccinated from 1994 using “Pneumabort-K + 1b” (Fort Dodge, USA) at the time intervals recommended by the manufacturer.

All horses were dewormed every three months. After weaning, the hooves were examined and any problems corrected on a six-weekly basis. All foals were treated symptomatically for any clinical disease.

2.1.2 FOAL SELECTION AND PROFILE

Forty pregnant mares were selected by the owner, about one month before their expected parturition date. Thirty healthy foals that were born to these mares between August and November 1993, were randomly singled out for the purpose of the investigation. These foals were monitored for the presence of respiratory disease and sampled on a monthly basis for one year. Prior to registration of the foal, it was identified as the offspring of a particular mare, while after registration, which occurred just after weaning, it was identified according to its “passport”. This “passport” included a full description of the foal with date of birth, sex, colour, markings and registration number as well as that of its dam and sire. A unique identification number (*e.g.* F 1 - 40 for foals and a corresponding number M 1 - 40 for dams) was allocated to each animal and was used to identify all written observations and samples pertaining to that animal. The foals and their dams that were used in this investigation are listed in Table 2.

2.1.3 OBSERVATIONS

During the monthly visits to the farm the following observations were made:

- Identification of the foal (Table 2).
- Collection of any data related to the clinical history of the foals in the study in the month preceding the visit. This included any change in management, such as weaning, clinical disease and treatment and any special procedures.
- Clinical and any other relevant findings were recorded on a standard form (Table 3).

Table 2: Foals in the investigation. Please note that numbers are not consecutive, as either the mare aborted, or had a stillbirth, or the foal was born too late to be included in this investigation.

Name of foal	Date of birth	Sex	Foal number	Name of dam	Mare number
Streamline	10/10/93	F	F1	Fleeting Beauty	M1
Star North	30/09/93	M	F2	Miss North	M2
Star Gazing	05/10/93	F	F3	Meteorite	M3
Another Chance	19/10/93	M	F5	Quelle Chance	M5
Satin Weave	11/11/93	F	F6	Lacemaker	M6
Ciragan	01/11/93	F	F8	Pink Palace	M8
Litterati	10/09/93	M	F9	Pictorial	M9
Radar Queen	12/09/93	F	F11	Waveband	M11
Mountain Dancer	23/10/93	F	F13	Anne O' Cape	M13
Starfish	08/09/93	F	F15	Venus Ear	M15
Maestranza	06/11/93	M	F16	Lady of Seville	M16
Exorcist	19/09/93	F	F17	Fred's Imp	M17
Fairy Wind	02/09/93	F	F18	Fairy Glen	M18
Truscott	23/09/93	F	F19	Roseland	M19
Unnamed*	15/09/93	F	F20	Orinoco Flow	M20
Fermoy	14/09/93	F	F22	Lillibet's Friend	M22
Smart Money	17/09/93	M	F23	Jamacan Money	M23
Sissinghurst	24/09/93	M	F24	Garden Gala	M24
Capadolia	25/09/93	M	F25	Scorching	M25
Presov	01/09/93	F	F26	Press On	M26
Cousin Kate	10/09/93	F	F29	Great Niece	M29
Best Company	06/11/93	F	F30	Mi Campanero	M30
Exeter	25/10/93	M	F31	Lady Adare	M31
Essex	22/10/93	F	F32	Peterbrough	M32
Charmed Circle	27/10/93	F	F33	Daring Beauty	M33
Egremont	18/09/93	M	F34	Lady of Substance	M34
Spielberg	07/10/93	M	F35	Take My Picture	M35
Crown Colony	22/10/93	M	F37	Protectress	M37
Hubble	23/10/93	M	F38	Parhelia	M38
Bethany	03/10/93	M	F39	Bishop's Glen	M39

* Euthanased at the end of 1994 due to poor leg conformation.

Table 3: The form used to record the findings of the monthly clinical examination and the samples (if any) taken for laboratory analysis.

No.	Foal's name	Rectal Temp.	Clinical signs	Nasal discharge	B	M	V	S	H
F 1	Streamline								
F 2	Star North								
F 3	Star Gazing								
F 5	Another Chance								
F 6	Satin Weave								
F 8	Ciragan								
F 9	Litterati								
F 11	Radar Queen								
F 13	Mountain Dancer								
F 15	Starfish								
F 16	Maestranza								
F 17	Exorcist								
F 18	Fairy Wind								
F 19	Truscott								
F 20	Unnamed*								
F 22	Fermoy								
F 23	Smart Money								
F 24	Sissinghurst								
F 25	Capadolia								
F 26	Presov								
F 29	Cousin Kate								
F 30	Best Company								
F 31	Exeter								
F 32	Essex								
F 33	Charmed Circle								
F 34	Egremont								
F 35	Spielberg								
F 37	Crown Colony								
F 38	Hubble								
F 39	Bethany								

B: nasophyngeal swab for bacterial and fungal isolation; M: nasaopharyngeal swab for mycoplasmal isolation;
V: nasopharyngeal swab for virus culture; S: serum for serology; H: heparinized blood for virus isolation.
* Euthanased at the end of 1994 due to poor leg conformation.

2.1.3.1 Clinical examination

Prior to the collection of samples, each foal was examined clinically. The following clinical parameters were assessed and any abnormalities recorded:

- Habitus of the foal. Scored from 1 to 4. A healthy foal was given a score of four; three was allocated if the foal was lagging a little behind the mother when running and if it was showing a mild depression and inappetance; a score of two was given to a foal that was inappetant, moderately depressed but still standing and being able to walk; and a score of one was given to a recumbent, severely depressed and inappetant foal. Any excitement or other factors just prior to sampling that may have caused a temporary elevation in rectal temperature, respiratory and heart rates was also recorded.
- Respiratory rate measured per minute on a resting foal.
- Heart rate measured per minute by heart auscultation.
- Rectal temperature.
- Quantity and quality of nasal and ocular discharges, if any.
- Size of the palpable lymph nodes of the head.
- Presence of any oral lesions.
- Auscultation of the thorax to detect any abnormal sounds.

2.1.4 COLLECTION OF SPECIMENS

2.1.4.1 Mares

During September 1993, 10 ml of blood was collected from forty mares in red-capped Vacu-test tubes without anticoagulant within one month of their expected parturition date. This was to establish the serum antibody profiles to the viruses that affect the respiratory system and if there was a correlation between the antibody levels in the mares and those of just after suckling for the first time in their foals.

2.1.4.2 Foals

Blood specimens for serology taken before and after suckling for the first time:

Approximately 5 ml of blood were collected, in red-capped Vacu-test tubes without anticoagulant (Radem Laboratory Supplies, South Africa) from 17 of the foals (F2, F3, F8, F9, F13, F17 - F19, F20, F22 - F26, F30, F31, and F37) prior to suckling and all 30 of the selected foals after suckling for the first time.

Monthly collection of specimens:

The following specimens were collected from most of the selected 30 foals monthly from 21/09/1993 until 02/08/1994:

- Ten millilitre blood samples were collected in plain, sterile Vacu-test tubes via jugular venipuncture using a 19G vacutainer needle (Becton Dickenson, England) and bleeding cuff.
- Nasopharyngeal swabs:
  - One in viral transport medium.
  - One in bacterial transport medium.
  - One that was directly streaked onto mycoplasma culture medium (see below).

Some of the foals were not yet born during the first two samplings, namely; foals F1 - F3, F5, F6, F8, F11, F13, F16, F19, F20, F22, F24, F25, F29 - F35, F37 - F39 on 23/09/1993, and foals F3, F 30 and F33 on 26/10/1993. Foals F34 and F35 were in KwaZulu-Natal and Western Cape Provinces until January 1994. During the later samplings, it was not possible to sample all the foals as they were too excitable and sampling would have caused severe trauma. Those not sampled were: F17, F22, F24 and F35 on 24/02/1994; F2, F5 and F35 on 29/03/1994, F3, F8, F24, F31 and F38 on 2/04/1994; F31 on 4/05/1994; and F31 of 2/08/1994.

Foals showing signs of respiratory disease:

The same specimens as indicated in the paragraph above were also collected, prior to antibiotic therapy, when the foals showed one or more of the following clinical signs:

- A fever equal to, or higher than, 38,5 °C or one degree higher than their own base-line rectal temperature.
- Abnormal nasal discharge. This included a copious serous, mucoid, mucopurulent or purulent nasal discharge.
- Acute cough.
- Abnormal respiratory noises of acute onset.
- Presence of vesicular or ulcerative lesions in the oral cavity.

Additionally, a 5 ml blood specimen was collected in sterile, Vacu-test tubes containing heparin (Radem Laboratory Supplies, South Africa) via jugular venipuncture from all foals that had a fever equal to or higher than 38,5 °C, or one degree higher than their base-line temperatures. Blood specimens were only taken from foals in their first two months of life when their rectal temperatures were equal or greater than 39,1 °C. The following foals were sampled: on 23/09/1993, F9 and F17; on 26/10/1993, F2, F3, F32, F35 and F37; on 23/11/1993, F1, F2, 3, F5, F6, F8, F13, F16, F19, F20, F23, F24, F25, F29, F30, F33, F38 and F39: on 14/12/1993, F2, F3, F6, F8, F9, F15, F16, F17, F19, F20, F24, F25, F29, F30, F31, F38 and F39; on 20/01/1994, F1, F2, F3, F5, F6, F8, F9, 11, F13, F15, F18, F19, F20, F23, F26, F30, F31, F33 and F38; on 24/02/1994, F2, F3, F16,F20, F30, F31, F32, F33, F34, F35 and F37; on 4/05/1994, F3, F6, 30 and F38; on 6/06/1994, F8 and F16; on 27/06/1994,

F5, F6, F9, F11, F13, F16, F19, F24 and F25; on 2/08/1994, F24, F25 and F39; on 8/09/1994, F13; on 13/10/1994 F6 and F38.

2.1.4.3 Nasopharyngeal swabs

For virus isolation:

The modified method of Bryans *et al*., (1967) was used (Studdert *et al*., 1970). The suckling foals were restrained by a person who held their heads between his/her chest and hands. The weaned foals were restrained with a nose twitch. A sterile cotton gauze swab attached to a stainless steel wire 40 cm in length, was placed into one of the nostrils, moved gently through the nasal cavity until the nasopharynx was reached (usually when resistance to movement of the swab occurred), when it was gently rubbed against the mucosa for approximately one minute and removed. The steel wire immediately above the swab was cut off using the pliers and the swab was placed into a 20 ml MacCartney bottle containing 5 ml of phosphate buffered saline (PBS) pH 7,4 with 3 % foetal calf serum (FCS) to stabilize the virus and containing 5 mg gentamicin (Genta 50, Phenix) and amphotericin B (Fungizone, Squibb Laboratories) to prevent multiplication of most bacteria and fungi (see Figure 5).

For bacteria (including mycoplasma) and fungus isolation:

Commercially available sterile “mare endometrial” swabs (Clinical Science Diagnostics, South Africa) were used. They consisted of two sheathed cotton-tipped swabs 65 mm long packed with a plastic tube containing Stuart's bacteriological transport medium. One sheathed cotton swab was placed into the nasopharynx of the foal under restraint, exsheathed, rubbed against the nasal mucosa for five seconds, and removed from the

Figure 5: Cutting off a swab into a MacCartney bottle containing viral transport medium.

nasopharynx of the foal (Figure 6). Immediately after collection, on the farm, the swab was streaked onto both Hayflicks (Hayflick, 1965) and Chalquest's agar (Chalquest, 1962) for the isolation of mycoplasmas. Both agars contained 0,5 g/l of ampicillin (Ampimac suspension, Hexal Pharma SA (Pty) Ltd, South Africa) to inhibit penicillin sensitive bacteria. It was then broken off into a plastic tube containing Hayflick's broth (Hayflick, 1965). The other swab was inserted through the plastic sheath, rubbed against the mucosa of the nasopharynx, removed from the nasal cavity with the sheath and broken off into the plastic tube containing Stuart's bacteriological transport medium.

It must be noted, however, that nasopharyngeal swabs, are not suitable for the isolation of bacteria that cause lower respiratory tract infections. They can, however, be used in epidemiological surveys for resident bacterial populations and opportunistic bacterial, fungal and mycoplasmal pathogens in the nasopharynx (Martens *et al.*, 1981).

Provision was made in the protocol for the collection of TTA specimens from any foals that showed signs of lower respiratory tract disease using the method described by Hoffman & Viel (1997).

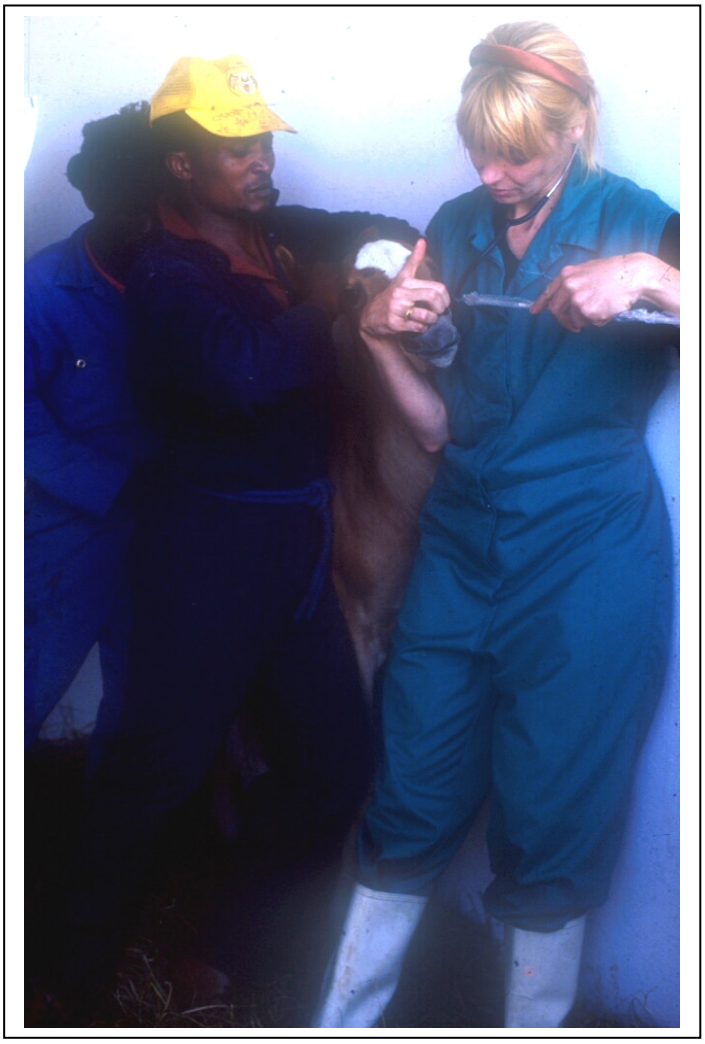

Figure 6: Preparing to swab the nasopharynx of a foal using a sterile "mare endometrial" swab.

2.1.4.4 Specimen handling on the farm

All specimens except for the agar plates were placed immediately after collection in a large polystyrene insulated container with five frozen refrigeration blocks and taken by road to the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria. A temperature of 4 °C was thus maintained for the period of transportation. The inoculated Hayflick's and Chalquest agars were placed in a candle extinction jar (approximate CO_2 content of 3 %) and transported at room temperature (about 26 °C) to the DVTD. These specimens all reached the laboratory within 8 hours after collection.

2.2 LABORATORY PROCEDURES FOR THE *IN VITRO* CULTURE OF VIRUSES

2.2.1 SPECIMEN PROCESSING IN THE LABORATORY

2.2.1.1 Nasopharyngeal swabs for viral isolation

All the nasopharyngeal swabs were vortexed for one minute, squeezed out using sterile forceps into the transport medium and the fluid filtered using a 0,22 µm disposable pore filter (Millipore, SA). The filtrate was stored at -70 °C in cryotubes until cell cultures became available. This usually occurred within a week of sampling.

2.2.1.2 Heparinized blood

Buffy coat was collected according to the method described by Stokes *et al*., (1991). Heparinized blood that was collected from foals with a fever reaction was centrifuged at 200 g for 20 minutes. After discarding the plasma, the cells were rinsed with PBS (see Appendix I) and re-centrifuged. This process was repeated once more. The red blood cells were haemolyzed by mixing the cells with an equal volume of sterile distilled water for one minute. Thereafter the osmotic pressure was restored with 10 x normal concentration of PBS. This mixture was centrifuged, the supernatant discarded and washed twice more. The leukocytes were then added to monolayer cell cultures.

2.2.2 HARVESTING OF PRIMARY EQUINE EMBRYONIC LUNG (EEL) CELLS

About a 10 cm^3 piece of lung was collected from a recently aborted equine foetus that was not aborted due to any known infectious disease. The lung was washed twice in calcium and magnesium free Phosphate Buffered Saline (PBS-)(see Appendix I). It was then cut up finely with a sharp sterile scissors and placed in a trypsinizing container containing a magnet and sufficient PBS- to cover the lung tissue. After stirring on a magnetic stirrer for 20 minutes the fluid was discarded, fresh PBS- added and the process repeated. Two hundred millilitres of a filtered 0,25 % solution of freshly made up trypsin (Difco 1:250) in PBS- were added to the lung tissue and stirred on a magnetic stirrer for 1 hour. Once removed from the magnetic stirrer the flask was allowed to stand for 2 minutes to allow the unbroken-down tissue to sediment out. The supernatant was carefully decanted into 20 ml MacCartney bottles and 1 ml of foetal calf serum (FCS) was added to each bottle to inactivate the trypsin. After

centrifugation at 550 g for 4 minutes the supernatant was discarded. Ten millilitres of Eagles Minimum Essential Medium (E-MEM) (Highveld Biologicals, South Africa), modified with Earle's salts and non-essential amino acids enriched with 0,295 % tryptose phosphate broth (Difco 1:120), sodium bicarbonate 2 g/l with gentamicin sulphate (Genta 50, Phenix, South Africa) at a concentration of 0,05 mg/ml with 10 % FCS was added and gently mixed. This was the maintenance medium used throughout this study and will from this point onwards be referred to as cell culture medium.

The cell mixture was repeatedly drawn up and squirted out using a glass syringe attached to a 15G needle. This was done to further separate the cells but in such a way that minimal damage was done to them. They were then collected in the syringe and divided between four 150 cm^2 culture flasks to which sufficient cell culture medium was added to cover the surface. The flasks were then tightly capped, laid flat in an incubator and cultured at 37 °C. Since tissue is usually still left in the trypsinizing container the process from trypsinization to the inoculation of culture flasks was repeated once more, with four more 150 cm^2 tissue flasks being prepared. As soon as the cells were firmly adhered to the tissue flask surface and there was clear division of cells, as observed under an inverted light compound microscope, the old virus culture medium was discarded and the cells were washed twice in PBS+ (PBS containing calcium and magnesium salts) (see Appendix I). This was to remove any dead cells, debris or connective tissue so that a true monolayer would form. Cell culture medium containing 10 % FCS was added to cover the surface and the flasks were incubated until a confluent monolayer was visible. The cell culture was then ready to be used in tests, to continue a cell line or for storage for later use. Primary cell lines usually can only be maintained for up to 8 to 10 passages before they loose their ability to divide at such a rate as to produce sufficient numbers of cells. Once this has happened the cell line was discarded and a new one propogated from the original stock.

2.2.3 PREPARATION OF MONOLAYER CELL CULTURES

Cell lines that were used were RK13 ATCC CCL13 (rabbit kidney13 American Type Culture Collection) for the isolation of EHV-1 (Stokes *et al.*, 1991), equine rhinoviruses (Mumford & Rossdale, 1980) and EAdV, Vero cells ATCC CCL81 (African green monkey) as most equine respiratory viruses except EHV-4 and EAdV-1 will grow in them, and primary EEL cells that are permissive to most respiratory viruses, including EAdV-1. None of these cell lines are known to support the growth of equine influenza virus. Preparation of both Vero and RK13 cell cultures was done as described by Burleson *et al*., (1992) and Lennette *et al*., (1988). The method used is briefly described.

Cell cultures stored in liquid nitrogen were rapidly defrosted at 37 °C (see Appendix I for the preservation solution used) and 1 ml was inoculated into 25 cm^2 sterile tissue flasks (Sterilab, South Africa) containing 10 ml of cell culture medium with 10 % FCS. After a confluent

monolayer had formed, the cells in the flasks were collected and split according to the split ratio of that particular cell line. One of these flasks was used to continue the cell line and the others for use in tests. The medium was decanted from the flasks and discarded. After washing twice using about 30 ml of PBS-, a further 20 ml of PBS- was added to each flask and placed in an incubator at 37°C for 5 minutes, excepting Vero cells which were incubated for 15 to 20 minutes. After decanting the PBS-, 20 ml of trypsin-versene solution (ATV) (see Appendix I) diluted 1:5 in water was added and gently spread over the entire surface of the flask by rocking the vessel side to side. The flasks were incubated at 37 °C for approximately 3 minutes and then checked to see if the cells had detached from the wall; if so cell culture medium with 10 % FCS was added to inactivate the trypsin, and if not they were incubated longer, for up to 10 minutes. The flasks were gently rotated to further seprate the cells and the contents were poured into 20 ml MacCartney bottles. Two millilitres of cell culture medium were added to the to each bottle to inactivate the trypsin. To sediment the cells the MacCartney bottle was centrifuged for 4 minutes at 650 g, the supernatant was discarded, and 10 ml of cell culture medium in 5 % FCS was added to the cell sediment and agitated using a glass syringe and 15 G hypodermic needle as previously described. Cells collected from a 150 cm^2 flask were made up to 50 ml and the cell suspension counted on a haemocytometer (see Appendix I). The cells were then diluted in E-MEM plus 5 % FCS to give the desired amount per 80 µl/well. Depending on the specific viral antigen being used in the test the concentration of cells used was 4,8 x 10^5/80 µl of Vero and equine lung cells, and 4 x 10^5/80 µl of RK13 cells.

2.2.4 VIRUS PROPAGATION

Two serial two-fold dilutions were made of each prepared sample and 0,2 ml of each dilution plus the original were inoculated into 10 ml glass test tubes containing a monolayer of cells and virus culture medium. The method used was adapted from Sugiura *et al*., (1988b). The cell cultures were placed in a rack that fitted in the rotating arm of an incubator set at 37 °C and allowed to gently turn (roller tube cultures). They were examined daily for CPE or rapid cell die-off using 200X magnification on an inverted compound light microscope (Nikon Corporation, Japan). Three tissue passages of 10 days each were carried out before a sample was considered negative.

2.2.5 VIRUS IDENTIFICATION

All cell cultures showing CPE (Figure 10) were examined using a transmission electron microscope (TEM) at 6000X magnification. The cells were collected by agitating the culture flasks that had a CPE of 90 % or more to remove all the cells from the flask surface. If cells were left, these were removed by treatment with trypsin. The cells were placed in 20 ml MacCartney bottles and centrifuged at 100 000 G for 45 minutes. The pellets were resuspended in 0,5 ml ammonium acetate, a drop of which was examined by negative staining using a TEM according to the method described by Studdert *et al*. (1970). Following

the identification to genus level by TEM the viruses were further characterized using virus neutralization tests (Mumford & Thomson, 1978a; Sugiura *et al*., 1988b).

2.3 LABORATORY PROCEDURES FOR THE *IN VITRO* CULTURE OF BACTERIA (INCLUDING MYCOPLASMAS) AND FUNGI

2.3.1 SPECIMEN PROCESSING IN THE LABORATORY

All nasopharyngeal swabs and, when taken, lymph node aspirates were checked for correct foal number or name and streaked out directly on the bacterial and fungal culture media after arrival at the laboratory. The period from sampling to plating of the specimens varied from three to eight hours. The same samples were used for both bacterial, excluding mycoplasmal, and fungal isolation. A separate nasopharyngeal swab was taken for mycoplasmal isolation.

2.3.2 AEROBIC BACTERIAL ISOLATION

The samples were streaked onto Petri dishes containing Columbia blood agar (Oxoid, Unipath, Basingstoke, England) containing 7 % horse blood, and MacConkey agar without crystal violet (Oxoid, Unipath, Basingstoke, England). The Petri dishes were incubated at 37 °C. The blood agars were placed in a candle extinction jar (approximately 3 % CO_2) and the MacConkey agars were incubated in air. The media were examined daily for 4 days for bacterial growth before being considered negative. Once growth was obtained, each different colony type was subcultured on blood and MacConkey agars in order to obtain pure colonies. These were identified according to the standard operating procedures of the laboratory. The identification methods used are well described in several textbooks (Carter & Cole, 1990; Holt *et al*., 1994; Koneman *et al*., 1995; Lennette *et al*., 1985; Quinn *et al*., 1994). Briefly, all bacterial colony types were subjected to the following tests:

- Description of the colony morphology on blood and MacConkey agars and whether it grew on MacConkey agar or not;
- Gram's stain to observe morphology and to determine whether they were Gram-positive or Gram-negative;
- Catalase test to detect the enzyme catalase;
- Oxidase test to detect cytochrome oxidase in the bacterial cell;
- Oxidative-fermentative (O/F) test for to test whether glucose is fermented or oxidized; and
- Motility test using the hanging drop method.

Based on the results of these tests the following biochemical tests or kits were used:

- *Gram-positive, catalase-positive, fermentative cocci that grew on MaConkey agar*: A commercial coagulase test, Staphytest plus (Oxoid, Basingstoke, England), and API

Staph (API Systems, BioMérieux sa, France) were used. These tests identified staphylococci to species level. Coagulase-negative staphylococci were considered part of the normal commensal microflora and not further identified. The non-pathogenic micrococci were distinguished from staphylococci by oxidation of glucose, sensitivity to bacitracin and resistance to lysostaphin.

- *Gram-positive, catalase-negative, fermentative cocci*: Lancefield cell-wall antigen typing using Streptococcal Grouping Kit (Oxoid, Basingstoke, England), fermentation of different peptone-based sugars, bile-aesculin and salt tolerance tests were used to identify the streptococci and enterococci to species level. However, alpha-haemolytic cocci that did not grow on MacConkey, did not type with the Lancefield cell-wall typing scheme and were optochin resistant were considered to be part of the *Streptococcus viridans* group and were not further identified. Bacteria in the latter class that were optochin sensitive were considered to be *Streptococcus pneumoniae*.
- *Aerobic, Gram-positive, catalase-positive, motile, fermentative, spore-forming rods*: They were considered to be *Bacillus* species and were not identified further.
- *Aerobic or faculatively anaerobic, catalase-positive, fermentative, non-sporing rods*: They were considered to be *Corynebacterium* species and were not identified further.
- *Gram-positive, anaerobic or faculatively anaerobic, non-sporing*, *pleomorphic bacteria with rudimentary branching*: These bacteria were considered to belong to the genus *Actinomyces* and were further identified using bile aesculin hydrolysis, nitrate reduction and sugar fermentation tests.
- *Pink, mucoid colonies that were Gram- and catalase-positive, non-motile and unreactive on the oxidative-fermentation test*: They were considered to a presumptive *R. equi.* The identification was confirmed by performing a CAMP test on sheep blood agar. This bacterium causes enhanced haemolysis of the haemolysins of *S. aureus* and inhibits haemolysis by the haemolysins of *Corynebacterium pseudotuberculosis.*
- *Gram-negative, oxidase-negative, rods that grew on MacConkey agar*: They were considered to be a member of the family *Enterobacteriaceae* and were identified to species level using motility test agar, triple sugar iron (TSI) slants, rapid spot Indole test using Kovac's reagent and API 10S (API Systems, BioMérieux sa, France).
- *Gram-negative, oxidase-positive coccobacilli* were futher identified using Microbact 12A and 12B for Gram-negative bacilli (Microgen Products Ltd., Surrey, UK) or API 20NE (API Systems, BioMérieux sa, France) and additional sugar fermentation tests if necessary.
- *Gram-negative, oxidase-negative, penicillin-resistant cocci that grew on MacConkey agar without crystal violet*: They were identified as *Acinetobacter* species. Their ability or lack of ability to ferment glucose was used to distinguish the species.

- *Gram-negative, oxidase positive, non-fermentative cocci*: They were further identified to genus level based upon their ability to grow on MacConkey agar without crystal violet and sensitivity to penicillin.

2.3.3 MYCOPLASMAL ISOLATION

The methods used are in the standard operating procedures of the Onderstepoort Veterinary Institute (Jacobsz, undated) The nasopharyngeal swabs for mycoplasmal isolation were plated out onto Hayflick's (Hayflick, 1965) and Chalquest's agars (Chalquest, 1962) on the farm and were transferred to a 5 % CO_2 in an air incubator set to 37 °C with 100 % humidity. The swabs in the Hayflick's broth were vortexed for 1 minute, the fluid collected in a disposable tuberculin syringe (Promex, South Africa) and filtered through a 0,4 µm microfilter (Millipore, South Africa). The filtrate was streaked onto Hayflick's and Chalquest's agars that were incubated as for the agar media. All cultures were examined on days 2, 5, 7 and 9 of incubation under a stereomicroscope at 4X magnification for the presence of typical "fried egg-like" colonies. Suspect colonies were cut out of the agar with a sterile scalpel blade and plated onto Hayflick's and Chalquest's agars to purify them. Immediately after detection, fungal contaminants were cut out of the agar and discarded. All mycoplasmas were recorded as being present, but were not identified further. They were stored at –70 °C (Randolph *et al*., 1993). Since specific antisera was not available, mycoplasmal isolates were not further identified.

2.3.4 FUNGAL ISOLATION

The culture and identification methods used have been well-described by Campbell & Stewart, (1981) and Rippon, 1982. After streaking the samples onto Petri plates containing Sabouraud's dextrose (SDA) (Oxoid, Unipath, Basingstoke, England) and Mycobiotic (MBA) (Difco laboratories, Detroit, USA) agars, they were incubated in air at 22 °C. The plates were examined every three days for colony growth for a period of one month. Each different colony type so obtained was sub-cultured onto SDA. Filamentous moulds were identified morphologically by the morphology and colour of both the upper- and under-surface of the colonies and by the microscopic characteristics of their fruiting bodies. The colonies were prepared for microscopic examination by lightly pressing a piece of clear adhesive tape onto a colony and placing the tape onto a microscope slide containing a drop of lactophenol cotton blue (see Appendix I). These were examined at 500X magnification under a light microscope with a lowered condensor and decreased substage diaphragm to improve contrast. Yeasts were identified using the germ tube and sugar assimilation (API 20AUX, API Systems, BioMérieux sa, France) tests.

2.4 VIRAL SEROLOGY

2.4.1 SPECIMEN PROCESSING IN THE LABORATORY

After arrival at the laboratory, the clotted blood samples were stored at 4 °C for 24 hours, to ensure maximal clot retraction and then centrifuged at 650 g for 10 minutes. The serum (supernatant) was pipetted using sterile Pasteur pipettes into 1,8 ml labelled cryotubes (Highveld Biological Products, South Africa) and frozen at –20 °C. The cryotubes were identified with the number of the foal and date of collection. Serological examination of the specimens was only performed after all the specimens had been collected.

2.4.2 SEROLOGICAL METHODS

All the stored sera were batch-tested in order to ensure standardization and for economical reasons. Using standardized antigen from reference viral strains, serological tests were performed and interpreted as laid out in Table 4. With the exception of the haemagglutination inhibition test (HIT) used to detect antibodies to EIV all tests were routinely done in the DVTD. Included in this chapter is a brief description of the methods used. The detailed methods are described in Appendix II.

Table 4: Serological tests that were used to detect antibodies to five viruses that are known to cause infectious respiratory disease in horses.

VIRUS	SEROLOGICAL TEST
Equid herpesvirus 1 (EHV-1)	CFT (Doll *et al*., 1953; Thomson *et al*., 1976) SNT (Burleson *et al*., 1992)
Equid herpesvirus 4 (EHV-4)	CFT (Sugiura *et al*., 1987) SNT (Thomson *et al*., 1976)
Equine influenza virus (EIV)	HIT (Sugiura *et al*., 1987)
Equine rhinovirus 1 and 2 (ERV-1 and ERV-2)	SNT (Senne *et al*., 1985)
Equine adenovirus (EAdV-1)	SNT (Kamada, 1978)

CFT = Complement fixation test; SNT: Serum neutralization test; HIT: Haemagglutination inhibition test

2.4.2.1 Haemagglutination inhibition test

The HIT was selected because haemagglutination antibody levels correlate well with immunity to equine influenza (Mumford *et al*., 1983) and the test is relatively easy to standardize. It detects antibodies at 10 days post-primary infection and 7 days after re-infection, but titres decrease rapidly thereafter (Hannant *et al.*, 1988). The HIT detects both IgM and IgG classes of haemagglutinating antibodies. Since the test is known for its day-to-day variation, it was repeated four times and only when reproducible results were obtained within a two-fold variation were they accepted as a unit.

Test procedure:

The test antigen was prepared by growing A/equine 2/Newmarket/79 (H3N8) in 10 to 11-day-old specific pathogen free embryonated hens' eggs. After three days the virus was harvested and concentrated by collecting allantoic fluid in a sterile container, mixing it with an equal volume of cold ether and retaining the aqueous layer. The virus titre was then determined by performing a haemagglutination titration in "V" bottomed 96-well microtitre plates with the highest dilution causing agglutination being considered to be one haemagglutination unit (HAU). Blood from healthy chickens was collected in Alsever's solution in a 1:4 concentration and allowed to stand for two days. A 0,5 % washed erythrocyte suspension was used for the test. After the test serum was inactivated at 56 °C for 30 minutes, it was added in a proportion of 1:10 to a 5 % chicken erythrocyte suspension in PBS to remove non-specific haemagglutinating activity. Using 0,025 ml micropipettes, and working in duplicate, two-fold serial dilutions from 1:10 to 1:640 were made of each serum to be tested. Four HAU /0,25 ml of virus was added to each well. A control to examine for non-specific haemagglutination was made by diluting the serum as for the test, but adding PBS instead of virus. After an incubation period of 30 minutes on a shaker at room temperature, 0,5 % of a washed chicken erythrocyte suspension was added to each well and allowed to settle at 4 °C for about an hour or until the cell control had settled. For each test-batch a known positive serum and known negative serum were included as well as a cell control containing only PBS and chicken erythrocytes. A virus back titration was also included to confirm that the correct virus titration was used. The HI titre was read as the reciprocal of the highest dilution of serum that completely inhibited haemagglutination.

2.4.2.2 Serum neutralization test

The SNT is based on the principal that when a specific immune serum is added to its corresponding virus, the virus is rendered non-infective or "neutralized". It is one of the few tests available that can measure protective antibodies. The microtitre method using a constant virus-varying serum concentration method was used to detect neutralizing antibodies to EHV-1 (Thomson *et al*., 1976), EHV-4 (Burleson *et al.*, 1992), EAdV (Kamada, 1978), ERV-1 and ERV-2 (Senne *et al*., 1985). Of the different neutralization tests this test has the advantage that there is only a small change in the antibody titre with a large change in viral concentration, requires a small viral concentration, can be tested over a wide range of serum dilutions and is economical in the use of reagents. The method used was the same for all the viruses tested. This test is routinely performed in the Virology Laboratory in the DVTD.

Test procedure:

Aseptic techniques and thoroughly washed and sterilized equipment were used at all times to avoid contamination and toxicity of the cell cultures. All the work was carried out in a laminar flow cabinet that had been regularly serviced. Viruses were propagated in permissive tissue cultures, namely EHV-1, EHV-4, and EAdV-1 were grown in primary EEL, ERV-1 in Vero and

ERV-2 in RK13 cell cultures. These cells were also used in the relevant SNT. To ensure that the correct viral concentration was used in the test, a viral titration was performed in triplicate as described in Appendix IV. One tissue culture infective dose$_{50}$ ($TCID_{50}$) of virus was taken as the virus dilution required to kill 50 % of the cells using the method of Reed & Muench (1938, cited by Lennette, 1969).

All the test sera were prepared by diluting them in PBS+ and then heating to 56 °C for 30 minutes to inactivate non-specific viral inhibitors. Using cell culture medium with 5 % FCS as diluent, serial 2-fold falling dilutions of serum, from 1:10 to 1:1280, were prepared in duplicate in flat-bottomed, 96-well microtitre plates with lids. The test virus was then added to each well at 100 $TCID_{50}$/80 µl. In each test batch a cell control and a virus back titration was included. The virus back titration was prepared by using four serial ten-fold dilutions of 100 $TCID_{50}$ of virus to be tested. After cell culture medium and 5 % FCS was added to the plates, they were incubated for one hour at 37°C in a 5 % CO_2 in air incubator. After incubation 80 µl of the required cells were added to each well. The plates were sealed and incubated at 37°C in a 5 % CO_2 in air incubator.

The progress of the CPE was read daily using the 20X objective of an inverted compound microscope (Nikon, Tokyo, Japan) and recorded in a book on a 0 to 4 scale with 0 being no infection of cells, 1, 2, 3 and 4 being approximately 25, 50, 75 and 100 % infection of the cells respectively. The end point was when the viral control showed mostly a reading of 4 at a dilution of 10^{-1} or less and 2 at the dilution of 10^{-2}, and the cells in the cell control appeared healthy. The antibody titre was expressed as the reciprocal of the highest dilution of serum that gave 50 % protection as calculated by the method of Reed & Muench (1938, cited by Lennette, 1969).

2.4.2.3 Complement fixation test

In this test the lytic ability of complement in the complement cascade reaction was used to detect immune complexes. The indicator system used was sheep erythrocytes (SRBC) and anti-sheep erythrocyte hyperimmune serum (amboceptor). In a positive sample complement binds to immune complexes made by the specific antigen:antibody reaction and thus none is left over to bind the erythrocyte-anti-erythrocyte antibody complex (haemolytic system). In a negative sample complement binds to the erythocyte-anti-erythrocyte antibody complex causing complement-mediated haemolysis. This test was used to detect complement fixing antibodies against EHV-1 and EHV-4 (Thomson *et al.*, 1976) that consist of IgM and some subclasses of IgG. The test procedure followed was the standard operating procedure used in the DVTD (Stylianides, 2000). An example of CFT test plate is given in Figure 7.

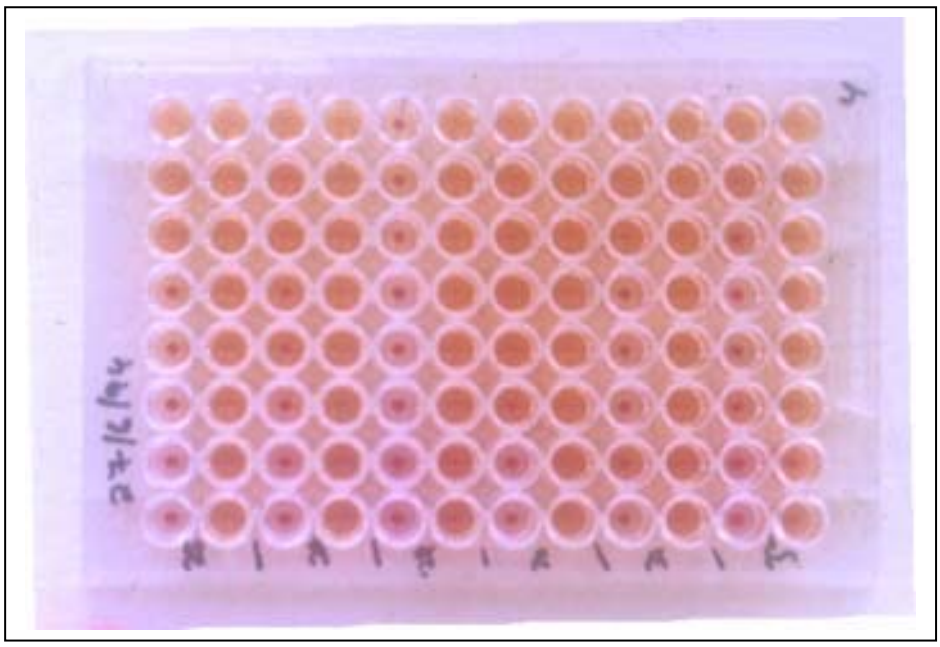

Figure 7: The presence of complement fixing antibodies to EHV-4 in a microtitre CFT.

Test procedure (for the detailed method refer to Appendix II):

The viral concentration used in the test was determined by a "checkerbox" titration method where serial dilutions of the virus are tested together with serial dilutions of serum known to have high levels of antibodies. The highest dilution of antigen with the highest dilution of serum where 50 % haemolysis occurred was considered to be one antigen unit.

The sera to be tested were diluted 1:5 in PBS+ and incubated for 30 minutes in a waterbath at 56 °C to inactivate anti-complementary factors present.

Each test was performed in duplicate. With each test batch a complement back titration, using 2, 1,5, 1 and 0,5 units of complement, was done to ensure that the correct concentration of complement was used. Also included in each test batch were known positive and negative sera. Two rows of serial two-fold serum dilutions were made from 1:5 to 1:640 in veronal buffered saline (VBS) (refer to Appendix I) in a "U" bottomed, 96-well microtitre plate and 25 µl of two units of viral antigen were added to the first row of test wells but not the second row as these were used to test for anticomplementary activity of the sera. These received 25 µl of VBS in the place of the viral antigen. Twenty-five microlitres containing twice the minimum haemolytic dose (MHD) of guinea pig complement were added to each well and the plates were sealed and incubated overnight at 4°C. The haemolytic system was prepared by making a 2 % solution in VBS of 3 to 5-day-old washed SRBC that was preserved in Alsever's solution (See Appendix I). This was then added to an equal volume of 1:2500 amboceptor (Virion). Fifty microlitres of the haemolytic system was added to each well, placed on a shaker and incubated at 37 °C for 20 minutes to ensure contact of virus-antibody complex with the haemolytic system. The test was considered to be complete

when the complement back titration results were as follows: 0,5 unit wells, no haemolysis (button formation); one unit wells, slight haemolysis; 1,5 and 2 unit wells, total haemolysis. The plates were then removed from the incubator and centrifuged for 10 minutes at 150 G. The antibody titre was expressed as the reciprocal of the highest dilution that caused a 50 % haemolysis of the erythrocytes.

CHAPTER THREE: RESULTS

3.1 CLINICAL EXAMINATION

3.1.1 OBSERVATIONS DURING MONTHLY VISITS

During the entire period of the study, no foal suffered from lower respiratory tract disease thus negating the need to take TTAs. The numbers of foals showing clinical signs of upper respiratory tract disease per sampling are depicted in Figure 8. As the environment was dusty, it was difficult to assess the significance of a serous nasal discharge. Foals that had an elevated rectal temperature of less than 1 °C, but no other signs of respiratory disease are excluded from Figure 8. All the foals had one or more bouts of upper respiratory disease during the period of study. Prior to weaning all but 3 foals (F8, F19 and F26) had exhibited clinical signs of upper respiratory tract disease. All of them, however, experienced clinical disease within two months post-weaning. Thirteen (43 %), 18 (60 %) and 17 (57 %) foals had, respectively, clinical signs of respiratory disease at weaning, one month and two

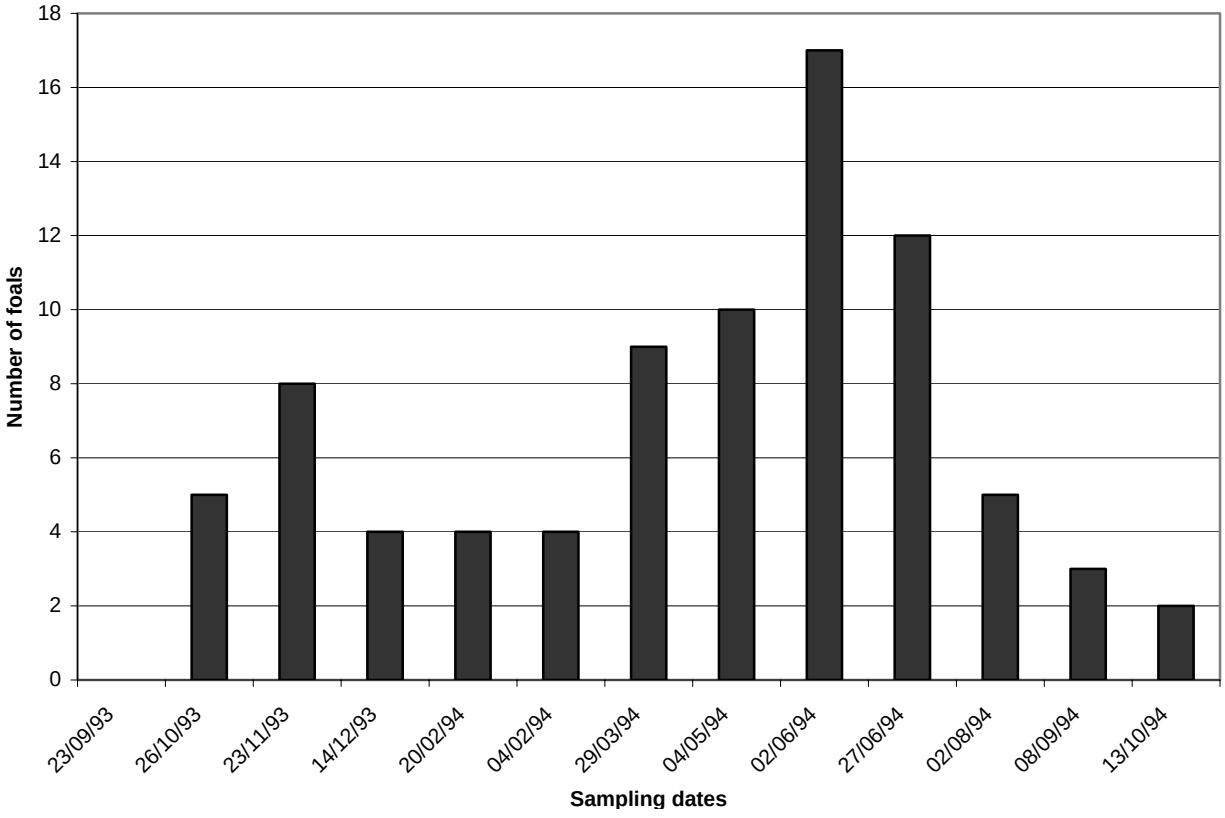

Figure 8: Number of foals showing clinical signs of upper respiratory tract disease. (Clinical signs that were considered significant included fever, swollen lymph nodes of the head region, particularly the mandibular lymph nodes, mucoid to mucopurulent nasal discharge, a cough and congested nasal mucosa).

months post-weaning. The weaning period was from May to June, when the foals were six months of age.

Two weeks preceding and during the 2/06/1994 sampling ten foals (F3, F6, F8, F13, F23, F26, F30, F34, F35, F39) manifested oral vesicles, erosions and ulcerations between 1 - 10 mm in diameter. This period coincided with the immediate post-weaning period and most of the other foals at the same time showed signs of respiratory disease. It was also at this time that four of the mares housed separately from the weanlings aborted as a result of EHV-1 infection. Aspirates of acute oral lesions from two of these foals were collected and submitted for scanning electron microscopy and tissue culture. No viruses could be isolated.

In Figure 9 the number of foals with an elevated rectal temperature are compared to those with both an elevated body temperature and clinical signs of upper respiratory tract disease. In the November 1993 and December 1993 samplings when many of the foals were neonates, a relatively high rectal temperature, up to 39 °C, was considered to be normal for this age group. Prior to weaning many foals had a fever but no clinical signs of respiratory disease. It was ascribed to excitement of capture and handling, biliary or diarrhoea in the foals (see 3.1.3). Fever accompanied by respiratory disease was more common in foals at foot than in weaned foals. Within 2 months after weaning the number of cases of upper respiratory disease tended to peak with most foals not exhibiting a fever reaction. Many of

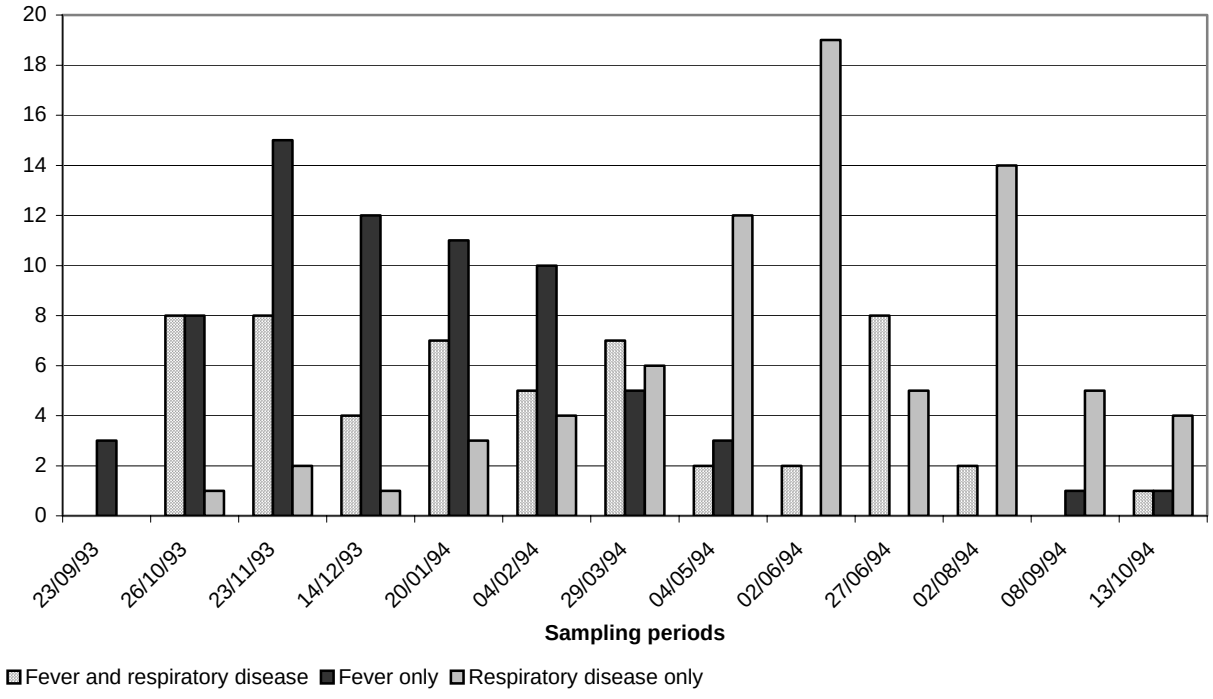

Figure 9: A comparison of the number of foals that showed fever and upper respiratory disease; fever only; and upper respiratory disease only.

the foals during this period had a swelling of the mandibular lymph nodes with some exhibiting abscessation of these lymph nodes.

3.1.2 TREATMENT OF FOALS WITH RESPIRATORY DISEASE

During the period of 05/05/94 to 14/05/94 foals F6, F11, F16, F17, F20 and F23 showed clinical signs of upper respiratory disease as well as fever and inappetance. They were all treated with trimethoprim combined with sulphadimethylpyrimidine (Amphoprim, Logos AgVet, South Africa) that was administered intramuscularly for 3 days at dosage rate of 8 ml. During the period 8/06/94 to 16/06/94 foals F5, F11, F15, F20, F23, F24, F29, and F37 were treated for upper respiratory tract disease with a penicillin and streptomycin combination (Pendistrep 20/20, Phenix, South Africa) administered intramuscularly at a dosage rate of 10 ml.

3.1.3 DISEASES NOT ASSOCIATED WITH THE RESPIRATORY TRACT

The most common non-respiratory tract disease reported was equine babesiosis caused by either *Theileria equi* or *Babesia caballi*. During the study period nine foals (F9, F13, F19, F22, F26, F29, F32, F33, and F37) between 1 to 5 months of age were treated for this disease with imidocarb dipropionate (Forray-65, Schering-Plough Animal Health, South Africa). The diagnosis was based on an elevated rectal temperature and icteric mucous membranes. No blood smears were examined, however, to confirm the diagnosis. Four of the foals suffered from a watery diarrhoea of 2 to 5 days in duration: F6 and F16 when 1 month of age, F9 at 3 months of age and F30 at 6 and 8 months of age. Faecal samples were collected for TEM for the presence of viruses, nematode egg counts per gram, and aerobic and anaerobic bacteriological culture. Faecal smears were stained to determine the presence of *Cryptosporidium parvum* using the modified Kinyoun acid-fast method (Alles *et al*., 1995). No diagnosis could be made in these cases. Another condition that occurred sporadically throughout the sampling period in individual foals without evidence of respiratory disease was conjunctivitis. One foal (F25), except during a rainy period just prior to weaning, constantly had conjunctivitis. This was most probably related to eye irritation as a result of the dusty conditions. During July 1994, foals F8 and F26 were injured and were treated with phenylbutazone (Equipalazone, Kyron Laboratories, South Africa) and procaine penicillin G (Compropen, Centaur).

3.2 THE *IN VITRO* CULTURE OF VIRUSES

Viruses were isolated from nasopharyngeal swabs taken from foals F3, F9, F15, F17, F19, F23, F24 and F38 during the 29/03/1994 and from F3 during 02/06/1994 sampling periods. The viruses were cultured from an equal number of colts and fillies. These viruses grew only on EEL (Figure 10) and were identified as a herpesvirus under TEM (Figure 11). These foals were approximately five months of age when the herpesvirus was isolated for the first time. No viruses were cultured from the heparinized blood samples.

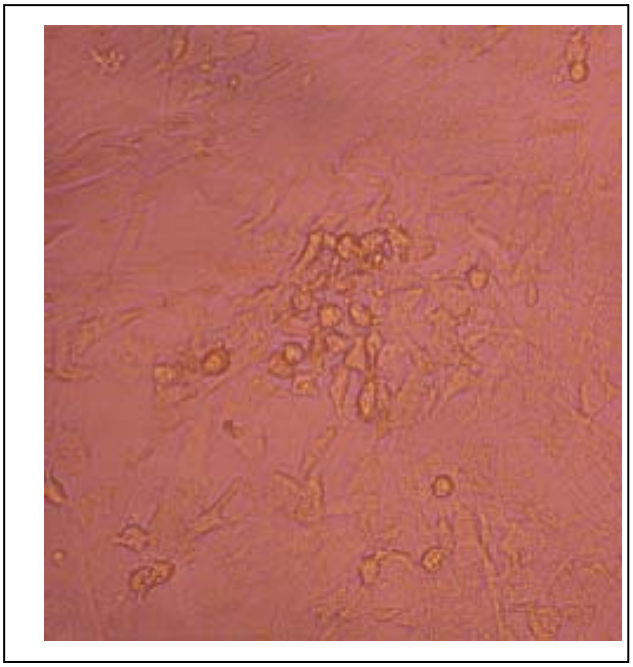

Figure 10: CPE due to EHV-4 in EEL tissue monolayer culture.

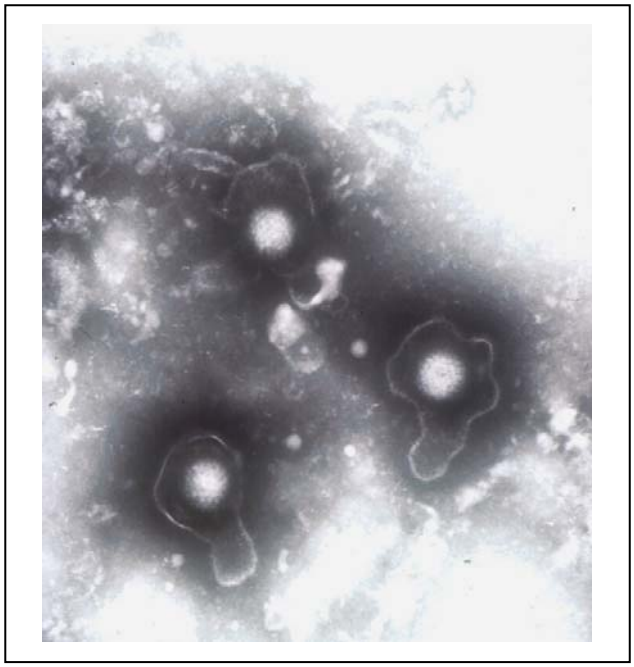

Figure 11: TEM 6000X magnification of EHV-4 isolated from foal F9.

The serum neutralization test (SNT) was performed on the isolated viruses using two different sera that contained high positive neutralization antibodies to EHV-1 and EHV-4 respectively. It was concluded that the viruses isolated were EHV-4 as they only grew on EEL and there was stronger neutralization of all the viruses isolated in the test which serum containing high antibody titres to EHV-4 was used.

3.3 THE *IN VITRO* CULTURE OF BACTERIA (INCLUDING MYCOPLASMAS) AND FUNGI

3.3.1 BACTERIA

Table 5 gives the number of samples taken per sampling period, the number of samples with only one bacterium species cultured and the proportion of Gram-positive to Gram-negative bacterial species. Table 6 shows the type and number of Gram-positive, and Table 7 the type and number of Gram-negative bacterial isolates per sampling period. Although the proportion of Gram-positive to Gram-negative bacteria varied, the Gram-positive bacteria predominated throughout the entire sampling period (Table 5). The most prevalent group comprised the coagulase-negative *Staphylococcus* spp. In Figure 12 the proportion of potential bacterial pathogens are compared with the normal bacterial microflora isolated from the upper respiratory tracts of these foals. A detailed table showing the results from individual foals is presented in Appendix III. The most common opportunistic pathogen isolated was *S. equi* subsp. *zooepidemicus*, comprizing 8,7 % of all bacterial isolates. It entered the nasal cavity of three of the foals (F3, F30 and F33) within one month after birth. During the 23/11/1993

sampling period it was found in 13 of the foals. The number of foals carrying this bacterium decreased slightly for the next five months and again increased at weaning, peaking in the 2/06/1994 sampling period, the time when most of the foals (73,3 %) had evidence of upper respiratory tract disease. It appeared to establish itself in foals F5, F6, F8, F20, F24, F26, F31 and F37. The other opportunistic bacterial pathogens that were isolated, included *S. aureus* (5,6 %), *A. equuli* (4,88 %), *S. intermedius* (0,71 %), *A. lignieresii* (0,53 %), *M. haemolytica* (0,36 %), *P. multocida* (0,18 %), *P. caballi* (0,09 %), *S. agalactiae* (0,09 %) and *K. pneumoniae* (0,09 %). No primary bacterial pathogens were cultured.

A comparison is made between the isolation of known opportunistic bacterial pathogens and clinical evidence of upper respiratory tract infections in foals in Table 8. it is clear from this table that although all the opportunistic pathogens tend to be more common in foals with upper respiratory tract disease, this effect is more marked for *S. equi* subsp. *zooepidemicus* throughout the test period and for *A. equuli* in the neonatal period. On the 29/03/1994 at the same time as when EHV-4 was isolated from the respiratory tract, foals that had evidence of bacterial disease of the upper respiratory tract were F20 from which a pure culture of *S. equi* subsp. *zooepidemicus* was obtained, F23 and F30 from which an almost pure culture of *S. equi* subsp. *zooepidemicus* and *A. equuli*, respectively, was obtained and F 31 from which a mixed culture of *S. equi* subsp. *zooepidemicus* and *A. equuli* was obtained. A pure culture of *Streptococcus equi* subsp. *zooepidemicus* was cultured from F32 on 4/05/1994. On 2/06/1994, this bacterium was also cultured from lymph node aspirates of F3, F18 and F26 and from the nasopharynx of 18 foals, 15 of which (F1, F3, F5, F6, F8, F18, F22 - F24, F26, F32, F34, F37 - F39) had clinical signs of upper respiratory tract disease. A similar result occurred in the next sampling when *S. equi* subsp *zooepidemicus* was isolated from 13 foals, 12 of which (F1, F6, F9, F11, F13, F19, F20, F24 - F26, F29 and F39) were suffering from upper respiratory tract disease. Two and 12 of the samples yielded no bacteria during the 23/11/1994 and 24/02/1994 sampling period respectively.

Table 5: A summary of bacteria isolated from the nasopharynx of the 30 foals sampled on a monthly basis.

Sampling dates	No of samples	Pure cultures	GP:GN
23/09/1993	5	0	4:1
26/10/1993	25	2	3.05:1
23/11/1993[#]	28	0	2.06:1
14/12/1993	28	0	1.29:1
20/01/1994	30	0	1.44:1
24/02/1994*	26	2	2.54:1
29/03/1994	27	0	1.86:1
02/04/1994	25	0	2.58:1
04/05/1994	29	1	2.52:1
02/06/1994	30	0	1.36:1
27/06/1994	30	0	2.25:1
02/08/1994	29	0	2.6:1

* 12 of the samples yielded no bacteria.
[#] Bacteria were not cultured from two of the samples
Gram-positive bacteria = GP; Gram-negative bacteria = GN

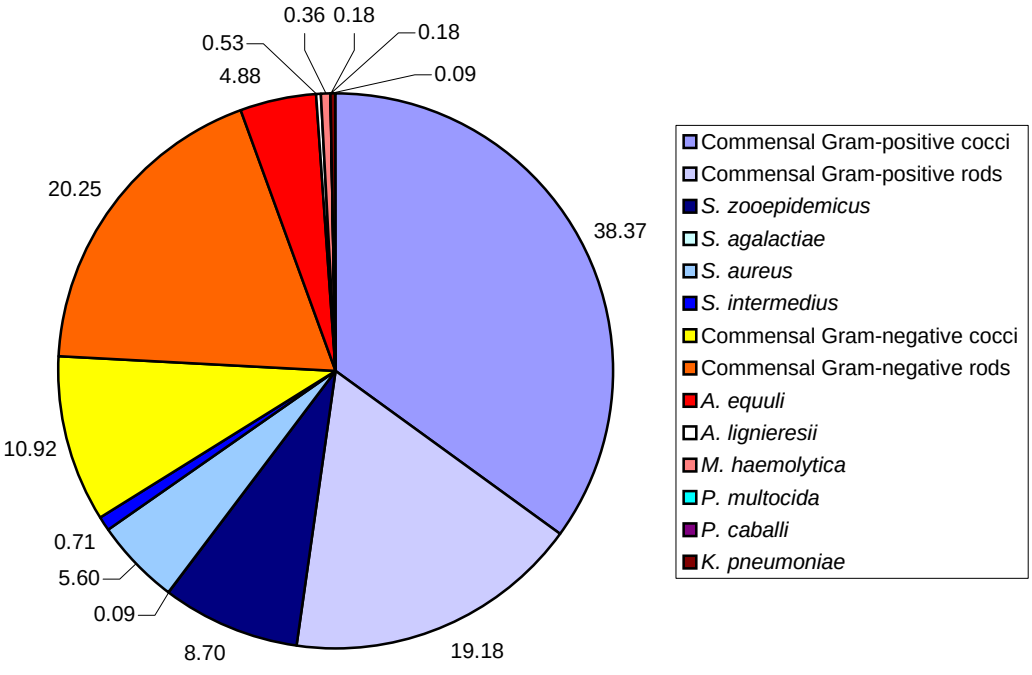

Figure 12: The proportion of potential bacterial pathogens to commensals of the upper respiratory tract throughout the sampling period.

Table 6: Gram-positive bacteria cultured from the 30 foals from 21/09/1993 until 02/08/1994.

	Sampling dates											
Bacteria	**21/09/1993**	**26/10/1993**	**23/11/1993**	**24/12/1993**	**20/01/1994**	**24/02/1994**	**29/03/1994**	**02/04/1994**	**04/05/1994**	**02/06/1994**	**27/06/1994**	**02/08/1994**
Aerococcus spp.	2	1	0	0	4	0	0	0	2	1	0	0
Agrabacterium radiatium	1	1	0	0	0	0	0	0	0	0	0	0
Bacillus sp.	2	7	8	13	13	3	4	3	3	17	6	2
B. cereus	0	0	0	1	2	0	0	0	4	4	2	1
Corynebacterium spp.	3	4	5	6	8	6	6	8	10	9	5	4
Enterococcus spp.	0	3	5	3	5	0	0	2	0	3	1	7
Kurthia spp.	0	1	0	0	1	0	0	0	0	0	0	1
Listeria spp.	0	0	0	0	1	0	0	0	0	0	0	0
Micrococcus spp.	4	1	0	1	0	0	0	1	0	0	1	6
Nocardia spp.	0	0	0	1	4	1	0	3	2	0	2	0
Oeskovia spp.	0	2	0	0	2	0	0	0	0	0	0	0
Pneumococcus sp.	0	1	0	0	0	0	0	0	0	1	0	0
Rhodococcus sp.	0	1	0	0	0	0	1	0	0	0	0	0
Streptococcus agalactiae	0	1	0	0	0	0	0	0	0	0	0	0
S. equinus	0	0	1	1	0	0	0	0	0	0	2	0
S. equi subsp. *zooepidemicus*	0	1	13	6	9	2	9	8	12	18	13	10
Staphylococcus aureus	0	1	1	2	2	3	7	12	16	6	5	8
S. intermedius	0	0	0	0	0	1	0	0	1	1	3	2
Staphylococcus spp.	3	28	14	22	28	12	27	37	27	17	33	49
Streptomyces spp.	1	1	2	2	0	2	2	1	3	9	2	0
Viridans streptococci	0	7	9	4	6	3	11	5	4	14	7	7
Total isolates	**16**	**61**	**58**	**62**	**85**	**33**	**67**	**80**	**84**	**100**	**82**	**97**

Table 7: Gram-negative bacteria cultured from the 30 foals from 21/09/1993 until 02/08/1994.

	Sampling dates											
Bacteria	**21/09/1993**	**26/10/1993**	**23/11/1993**	**24/12/1993**	**20/01/1994**	**24/02/1994**	**29/03/1994**	**02/04/1994**	**4/05/1994**	**2/06/1994**	**27/06/1994**	**2/08/1994**
Acinetobacter anitratus	0	2	0	4	0	0	0	0	0	0	2	1
A. wolffii	0	0	0	5	6	0	0	0	0	5	1	1
Actinobacilllus acetinomycetocomitans	0	0	0	0	1	0	1	1	0	1	0	0
A. equuli	0	3	7	14	9	2	10	1	4	5	0	0
A. lignieresii	0	0	1	1	3	0	1	0	0	0	0	0
Aeromonas hydrophila	0	0	0	0	0	0	0	3	2	0	2	2
Burkholderia cepacia	1	1	0	0	4	2	4	2	0	5	1	2
Enterobacter cloacae	0	0	0	0	0	0	0	0	0	2	0	0
Enterobacter sp.	0	0	0	0	0	1	0	0	0	0	0	0
Escherichia coli (rough)	0	3	0	3	3	0	0	0	0	5	4	2
Flavobacterium spp.	0	4	2	4	11	3	5	4	5	15	6	3
Klebsiella pneumoniae	0	0	1	0	0	0	0	0	0	0	0	1
Mannheimia haemolytica	0	0	0	1	0	0	0	0	1	0	0	2
Moraxella spp.	1	2	3	5	5	3	11	14	13	16	12	9
Neisseria sp.	0	0	0	0	0	0	1	1	1	0	0	1
Pantoea agglomerans	0	0	1	0	3	0	0	0	0	0	1	3
Pasteurella sp.	0	0	2	9	1	0	1	1	1	6	1	3
P. caballi	0	0	0	0	0	0	0	0	0	0	0	1
P. multocida	0	0	0	0	0	0	0	0	0	0	1	1
Proteus mirabilis	1	1	1	0	5	1	1	0	2	1	0	0
P. rettgeri	1	0	1	0	0	0	1	0	0	5	0	0
P. vulgaris	0	2	0	0	0	0	0	2	0	0	0	2
Pseudomonas maltophilia	0	1	0	0	0	0	0	0	0	0	0	0
Pseudomonas sp.	0	0	0	0	0	0	0	1	2	2	3	0
Serratia rubidea	0	1	0	0	0	1	0	0	0	0	0	0
Simonsiella sp.	0	0	0	0	0	0	0	0	0	2	0	0
Streptobacillus sp.	0	0	14	2	8	0	0	1	2	5	4	2
Total isolates	**4**	**20**	**33**	**48**	**59**	**13**	**36**	**31**	**33**	**75**	**38**	**36**

Table 8: The association of opportunistic bacterial pathogens of the upper respiratory tract and disease in foals.

	Sampling dates											
	23/09/1993	**26/10/1993**	**23/11/1993**	**14/12/1993**	**20/01/1994**	**24/02/1994**	**29/03/1994**	**02/04/1994**	**04/05/1994**	**02/06/1994**	**27/06/1994**	**02/08/1994**
A. equuli												
Upper respiratory tract disease	0	5	4	5	4	0	5	1	2	2	0	0
No upper respiratory tract disease	0	0	3	10	4	2	5	0	2	2	0	0
S. equi subsp. *zooepidemicus*												
Upper respiratory tract disease	0	0	9	3	3	1	8	4	8	15	12	7
No upper respiratory tract disease	0	1	3	6	4	1	1	4	6	3	1	3
Other opportunistic pathogens*												
Upper respiratory tract disease	0	1	1	2	1	3	1	4	5	3	4	3
No upper respiratory tract disease	0	1	1	3	1	1	3	4	1	2	3	3
No known pathogens												
Upper respiratory tract disease	0	3	2	0	4	1	0	3	3	2	2	3
No upper respiratory tract disease	5	15	4	5	10	14	5	6	3	0	6	7

* Other opportunistic pathogens included *S. aureus*, *S. intermedius, S. agalactiae*, *A. lignieresii, E. coli* and *M. haemolytica*

3.3.2 MYCOPLASMAS

Mycoplasma species were cultured from most of the nasopharyngeal samples. Exceptions were when mycoplasmal colonies could not be detected because the culture plates were overgrown with fungi. This occurred in about one third of the samples.

3.3.3. FUNGI

Table 9 gives the number of samples taken per sampling period and the numbers and percentage of samples that yielded no viable fungi, and Table 10 shows the number of fungal isolates per sampling period. A detailed table giving the results from each foal per sampling period is in Appendix IV.

A large variety of fungal species were cultured from the nasopharynx of the foals. *Aspergillus* spp. (25,2 %) and non-pathogenic members of *Cladophialophora* spp. (18,3 %) were the most common fungi isolated. Yeasts were the least common.

Table 9: A summary of the number of samples taken and the number from which no fungi where cultured.

Sampling dates	No. of samples	No. of no growths	Percentage no growths
21/09/1993	5	3	60,0
26/10/1993	25	8	30,8
23/11/1993	28	6	26,1
24/12/1993	28	5	17,9
20/01/1994	30	7	25,9
24/02/1994	26	5	19,2
29/03/1994	27	1	3,7
02/04/1994	25	5	17,9
04/05/1994	29	3	10,0
02/06/1994	30	3	10,0
27/06/1994	30	1	3,3
02/08/1994	29	1	3,3

In this study the number of fungal species isolated that may cause mycoses in horses were few: *A. fumigatus* (1.8 %) which can cause a fungal infection of the nasal cavity, guttural pouch and lungs (Ryan *et al*., 1992) and, *Trichophyton equinum* (0,37 %), *T. schoeleinii* (0,19 %), *T. tonsurans* (0,19 %) and *Microsporum gypseum* (0,19 %) that cause dermatophytosis (Shimozawa *et al*., 1997; Tanner 1982). The number of foals from which no fungi were cultured decreased with age, while the number of species cultured increased with age**.**

Table 10: A summary of the fungal species isolated from the foals.

Fungus	Sampling dates											
	21/09/1993	26/10/1993	23/11/1993	24/12/1993	20/01/1994	24/02/1994	29/03/1994	02/04/1994	4/05/1994	2/06/1994	27/06/1994	2/08/1994
Hypomycetes												
Arthrobotrys oligospora	0	0	0	0	0	0	0	0	0	0	0	5
Aspergillus fumigatus	0	2	0	3	0	2	4	7	4	7	1	1
A. flavus	2	2	2	1	0	3	2	0	1	1	3	3
A. glaucus	0	5	0	2	1	1	5	2	4	1	1	3
A. nidulans	0	0	0	0	0	0	1	0	0	0	0	0
A. niger	0	3	3	1	1	0	0	0	5	0	1	0
A. terreus	0	0	0	0	0	2	3	2	2	8	2	7
A. versicolor	0	1	0	1	0	1	2	2	1	1	1	0
Beauveria spp.	0	0	0	0	0	0	0	0	0	1	1	1
Botrytis sp.	0	0	0	1	0	0	0	0	0	2	1	0
Chaetomonium sp.	0	0	0	0	0	0	0	0	0	0	0	1
Chrysosporium spp.	0	1	0	1	1	1	1	3	2	1	0	2
Fusarium spp.	0	0	0	1	1	2	6	5	2	2	3	3
Humicola spp.	0	0	0	0	0	0	1	1	0	0	0	1
Microsporum gypseum	0	0	0	0	0	0	0	0	0	0	0	1
Monosporium spp.	0	1	1	1	1	0	1	1	0	0	1	0
Penicillium spp.	0	1	2	1	0	0	3	3	5	3	6	0
Scopulariopsis spp.	0	1	0	0	0	0	0	0	1	2	2	3
Sepedonium spp.	0	0	0	0	0	0	0	0	0	0	1	1
Synecephalstrum spp.	0	1	0	0	0	0	0	0	0	0	0	0
Torula spp.	0	0	0	0	0	0	0	0	0	0	0	2
Trichophyton ajello	0	1	0	4	3	0	0	0	2	0	0	0
T. equinum	0	0	0	1	0	0	0	0	0	1	0	0
T. meganinii	0	0	0	1	2	0	0	0	0	0	0	0
T. schoeleinii	0	0	0	0	1	0	0	0	0	0	0	0
T. rubrum	0	0	0	0	0	1	1	0	1	0	0	0
T. tonsurans	0	0	0	0	1	0	0	0	0	0	0	0
Verticillium spp.	0	0	0	0	0	0	0	0	0	0	0	2

[1]

[1] Table 10 is continued overleaf

Table10 continued: A summary of the fungal species isolated from the foals.

	Sampling dates											
Fungus	**21/09/1993**	**26/10/1993**	**23/11/1993**	**24/12/1993**	**20/01/1994**	**24/02/1994**	**29/03/1994**	**02/04/1994**	**4/05/1994**	**2/06/1994**	**27/06/1994**	**2/08/1994**
Dematiaceous fungi												
Alternaria spp.	0	1	3	8	3	8	7	5	4	5	5	5
Aureobasidium pullulans	0	1	1	0	0	0	0	0	0	0	0	0
Bipolaris spp.	0	0	0	0	0	0	0	0	2	0	0	0
Cladophialophora spp.	0	2	4	7	7	17	18	9	9	6	11	8
Curvularia spp.	0	0	1	2	0	1	2	0	0	0	2	0
Drechslera sp.	0	0	0	0	0	0	0	0	0	2	0	1
Epicoccum spp.	0	1	2	0	0	0	2	0	1	1	0	2
Exophiala spp.	0	0	1	1	0	0	2	1	0	1	0	1
Fonsecaea sp.	0	0	0	1	0	0	0	0	0	1	0	0
Helminthosporium spp.	0	0	1	0	0	1	0	0	0	1	0	0
Rhinodocladiella sp.	0	0	0	0	0	0	0	0	0	0	0	3
Zygomycetes												
Absidia spp.	0	0	0	1	0	1	1	2	4	5	6	4
Mucor spp.	1	4	1	2	3	0	0	2	1	1	1	0
Rhizopus spp.	0	1	2	3	2	2	1	3	0	4	3	1
Yeasts												
Candida spp.	0	2	1	2	3	0	1	2	1	2	1	0
Geotrichium spp.	0	1	1	1	0	0	0	0	2	0	0	0
Rhodotorula spp.	0	0	0	2	1	1	0	1	0	0	0	0
Trichosporon spp.	0	0	0	0	0	1	1	1	0	0	0	5
Total isolates	**3**	**32**	**26**	**49**	**31**	**45**	**65**	**52**	**54**	**59**	**53**	**66**

3.4 VIRAL SEROLOGY

The serum level of the colostrum-derived antibodies of each foal and the time it took for them to wane as well as the serological response to natural infection and/ or vaccination for the different viruses were measured. Mean values of the reciprocal of the highest dilutions serum that detected antibodies are listed in Table 11. The lowest and highest value for each sampling is given in brackets. Graphical representations of these results are given in Figures 13 to 28. The tables in Appendix V should be consulted for the antibody titres to the viruses of each foal.

The results of the means of the antibody titres for mares and their foals are depicted in Table 11 and Figure 13. Pre-suckling serum lacking antibodies was collected from 17 of the foals.

The antibodies of the foals were similar or slightly lower or higher than that of the mares. So-called pre-suckling antibodies to EHV-1 were noted in F1, F9 and F32, to EHV-4 in F1 and F32, to ERV-1 in F1, F9, F17 and F32, to ERV-2 in F1 and F17, and to EAdV in F3, F17 and F32. All the antibody titres to the listed viruses rose in the post-suckling sera of these foals. Antibodies to all the tested viruses in the post-colostrum serum were absent in 1 foal (F26).

Since colostrum-derived antibodies are broken down at a constant rate in the tissues of a foal, the so-called $T_{½}$, (i.e. the time it takes for antibody titres to wane) is proportional to the initial concentration of ingested antibodies (McGuire & Crawford, 1973). This breakdown of antibodies is best depicted by using the age of the foal and not the date of sampling as there was a 70-day gap in age between the youngest and the oldest foal (see Table 2). Once the levels of colostrum-derived immunity in the foals had waned their serum antibody levels were related more to the actual presence of virus circulating in a herd rather than age. Thus two graphs were used, one showing the breakdown of colostrum-derived antibodies related to age of the foal and the other depicting a rise in antibody titre related to date of sampling. In most of the samplings there was a wide distribution in antibody titres in the group of foals (Table 11), making it difficult to show any trends. However, trends were more easily recognised when the means of the results were depicted or when the antibody profiles of individual foals were studied. The mean antibodies titres to each virus tested are depicted in Figures 13 to 28.

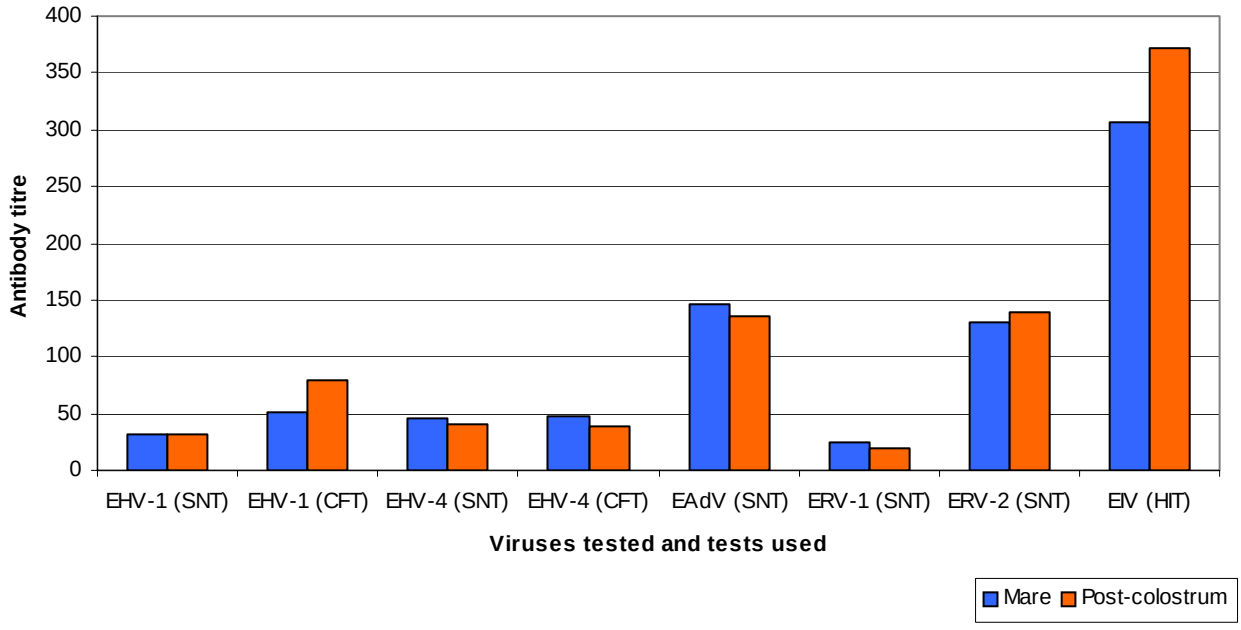

Figure 13: Mean antibody titres of thirty mares and their foals to five viruses.

Table 11: Mean antibody titres of the viruses tested in sera taken from 30 foals. The figures in brackets indicate the range of antibody titres per sampling period.

Serum Sampling	EHV-1 SNT	CFT	EHV-4 SNT	CFT	ERV-1 SNT	ERV-2 SNT	EAdV SNT	EIV HIT
Mares	31.13 (0-120)	50.83 (0-240)	45.50 (0-160)	48.39 (0-160)	149.3 (0-320)	24.83 (0-120)	132.30 (0-240)	131.33 (0-1280)
Foals								
Ages								
Pre-colostrum intake	5.50 (0-80*)	0 (0-80*)	5.00 (0-60*)	48.50 (0-60*)	0	0	0 (0-120*)	0
Post-colostrum intake	31.38 (0-120)	78.57 (0-360)	40.89 (0-160)	37.89 (0-160)	136.4 (0-320)	18.57 (0-80)	140.00 (0-320)	372.14 (40-1280)
1 month	13.83 (0-80)	41.83 (0-240)	26.00 (0-160)	24.07 (0-140)	86.60 (0-320)	12.41 (0-80)	96.17 (0-320)	320.30 (40-1280)
2 months	6.17 (0-60)	6.03 (0-40)	6.03 (0-40)	9.31 (0-80)	58.80 (0-320)	4.14 (0-20)	51.55 (0-160)	146.00 (0-240)
3 months	1.67 (0-20)	1.21 (0-10)	0.35 (0-10)	3.97 (0-20)	17.24 (0-160)	2.93 (0-40)	20.97 (0-120)	51.60 (0-160)
4 months	0	0.93 (0-20)	0	4.56 (0-60†)	6.79 (0-80)	1.07 (0-20)	10.40 (0-80)	18.00 (0-120)
5 months	0	1.38 (0-20)	0	2.59 (0-40)	1.38 (0-20)	0.86 (0-15)	9.31 (0-60)	14.67 (0-80)
Sampling dates								
24/02/1994	0	0	0	2.258 (0-60)	0	0.54 (0-15)	9.83 (0-80)	14.67 (0-80)
29/03/1994	0	1.67 (0-20)	0	1.613 (0-15)	0	0.833 (0-15)	8.50 (0-60)	9.50 (0-30)
4/05/1994	0	13.45 (0-60)	0.86 (0-15)	44.70 (0-160)	0	1.38 (0-20)	24.83 (0-320)	7.41 (0-20)
02/06/1994	2.00 (0-30)	22.45 (0-80)	7.41 (0-60)	46.03 (0-240)	0	6.38 (0-60)	23.79 (0-120)	12.80 (0-60)
27/06/1994	9.84 (0-40)	66.83 (0-1280)	23.33 (0-120)	76.50 (0-640)	0	5.67 (0-30)	23.50 (0-120)	32.50 (0-320)
2/08/1994	8.33 (0-60)	22.74 (0-80)	27.83 (0-120)	73.33 (0-160)	0	6.00 (0-30)	12.67 (0-60)	20.52 (0-120)
8/09/1994	7.83 (0-60)	13.87 (0-60)	15.17 (0-160)	20.00 (0-80)	0	8.83 (0-30)	17.00 (0-160)	40.38 (0-160)
13/10/1994	5.35 (0-30)	44.68 (0-160)	17.67 (0-60)	25.93 (0-240)	0	9.67 (0-40)	59.67 (0-320)	18.33 (0-320)

* The higher values are probably due to foals suckling prior to sampling (see text), † Foal F35, had early waning of antibodies and was susceptible at a younger age to EHV-4.

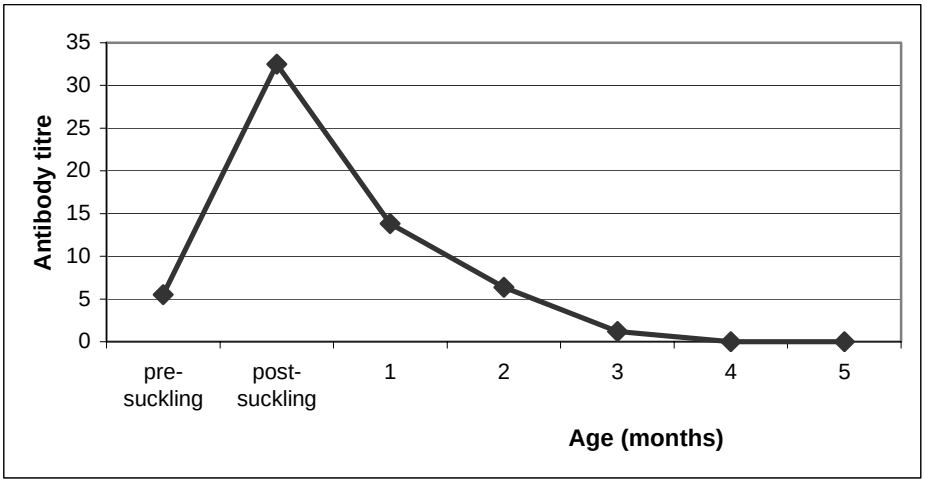

Figure 14: Mean SN colostrum-derived serum antibody titres to EHV-1 in the 30 foals.

Serum neutralizing (SN) and complement-fixing (CF) antibodies to EHV-1 were detected for up to 2 to 4 months of age with neutralizing antibodies lasting longer (Figures 14 and 15). The post-colostrum neutralizing antibody titres ranged from 0 to 1:120 and the complement-fixing antibodies from 0 to 1:360. In 10, five and three foals the colostrum-derived neutralizing antibody titres had waned by the time the foals were, respectively, 1, 2, 3 and 4 months of age. The colostrum-derived CF antibody titres had waned in six, nine, seven and three of the foals by the time they were, respectively, 1, 2, 3 and 4 months of age (Tables 14 and 15 in Appendix V).

Antibodies to EHV-1 starting rising when the foals were five to six months of age for the first time after maternal immunity had waned. The CF antibodies began to rise earlier but persisted for shorter periods than the SN antibodies (Figures 15 and 17).

The dates on which vaccination with Fluvac EHV4/1 Plus was administered are indicated on Figures 15 and 17 with black arrows. Complement-fixing but not neutralizing antibodies to EHV-1 were measured in 20 foals prior to vaccination with Fluvac EHV4/1. The values ranged from 1:10 to 1:60 with most having a titre of 1:15. A steep rise, however, in both CF antibody titres was only noted after the booster vaccination. These titres gradually declined until the last sampling. The SN titres only rose in three (F23, F24 and F35) of the foals after primary vaccination and in 16 of the foals after a booster was administered.

There was a more than a 4-fold increase in CF antibody titre in 14 of the foals during the 13/10/94 sampling when the foals were one year of age. This increase was unrelated to vaccination.

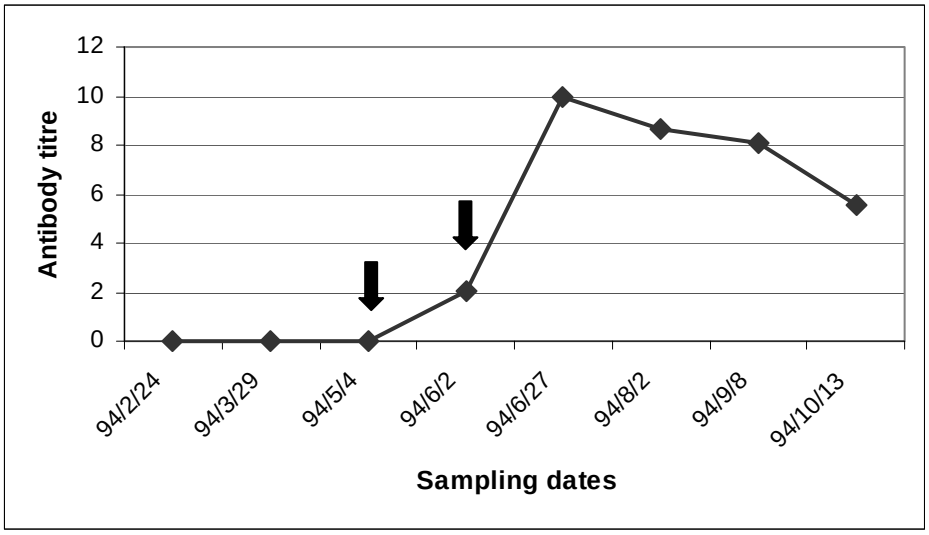

Figure 15: Mean serum SN antibody titres to EHV-1 in the 30 foals (The black arrows indicate when foals were vaccinated with Fluvac EHV4/1). Plus.)

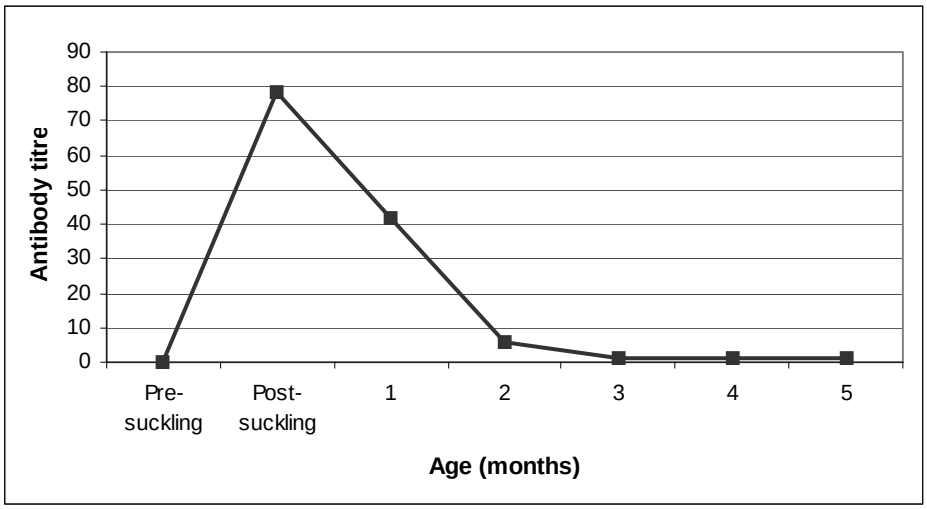

Figure 16: Mean CF colostrum-derived serum antibodies to EHV-1 in the 30 foals.

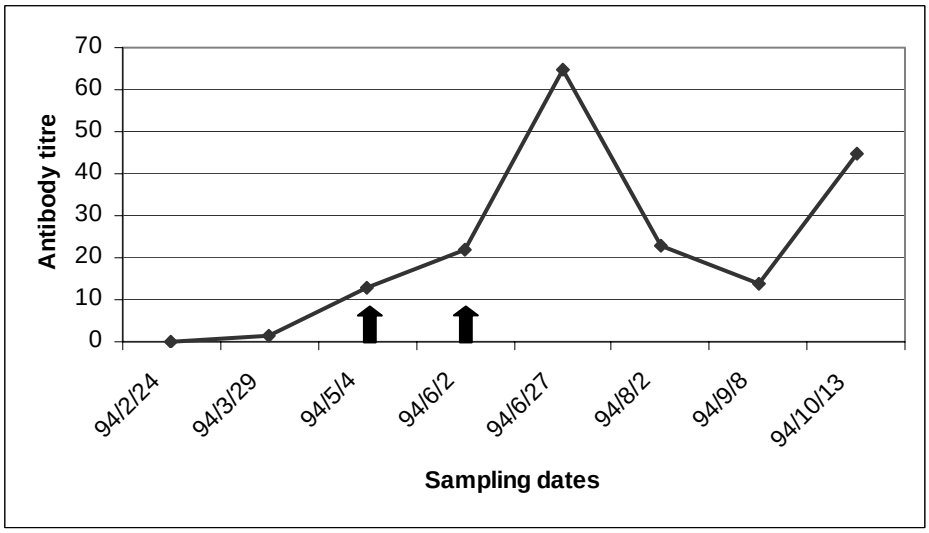

Figure 17: Mean serum CF antibodies to EHV-1 in the 30 foals (The black arrows indicate when foals were vaccinated with Fluvac EHV4/1 Plus.)

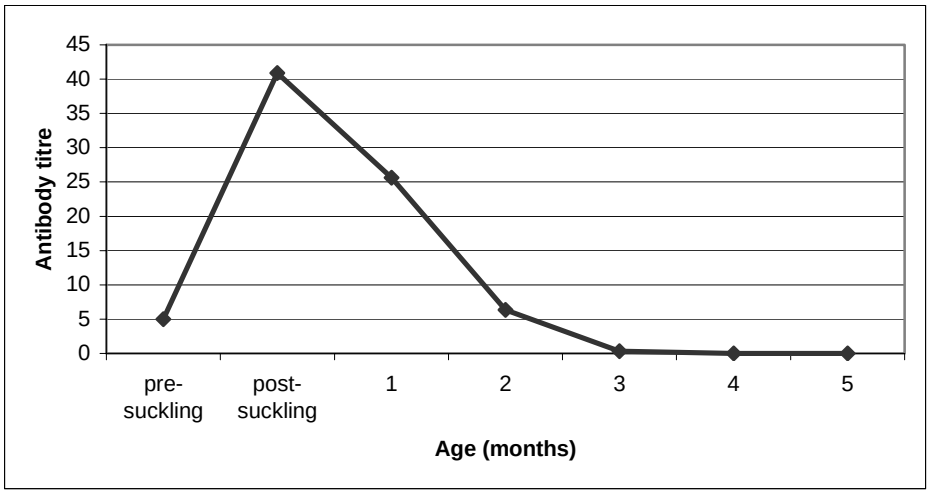

Figure 18: Mean serum SN colostrum-derived serum antibodies to EHV-4 in the 30 foals.

In the case of EHV-4, SN and CF antibodies were detected up to 2 to 5 months of age (Figures 18 and 20). The post-colostrum SN and CF antibody titres ranged from 0 to 1:160. Colostrum-derived antibodies to EHV-4 were immeasurable in two of the foals (F13 and F26). In eight, 10 and eight foals the colostrum-derived SN antibody titres had waned by the time the foals were, respectively, 1, 2 and 3 months of age. The colostrum-derived CF antibody titres had waned in seven, five, six, nine and one of the foals by the time they were, respectively, 1, 2, 3, 4 and 5 months of age (Tables 16 and 17 in Appendix V).

Complement-fixing antibody titres rose for the first time in three (F18, F20 and F35) of the foals on the 29/03/1994 sampling and 26 of the foals on the 4/05/1994 sampling. The SN antibody titres rose in two (F26 and F33) of the foals on the 4/05/1994 sampling and in seven of the foals on the 29/03/1994 sampling. After vaccination and especially after the booster was administered the SN and CF antibody levels in most foals rose steeply and antibodies were detected in many more foals (Figures 19 and 21). Seventeen of the foals had SN antibodies and 29 had CF antibodies on the 27/06/1994 sampling. From 2/6/1994 until the 13/10/1994 sampling periods individual foals had more than a 4-fold rise in antibody titre. They were F37 on 2/6/1994, F30 on 27/06/1994, F39 on 2/08/1994 and F16 and F17 on 13/10/1994. A rise in the SN antibody titre was noted at the 4/05/1994 sampling and reached higher levels in more of the foals at the samplings of the following two months.

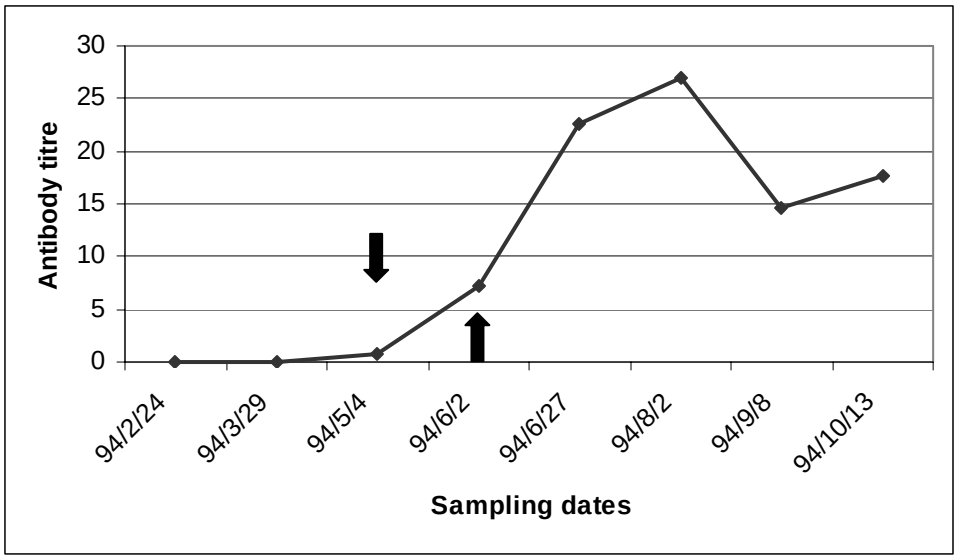

Figure 19: Mean serum SN antibody titres to EHV-4 in the 30 foals (The black arrows indicate when foals were vaccinated Fluvac EHV4/1 Plus).

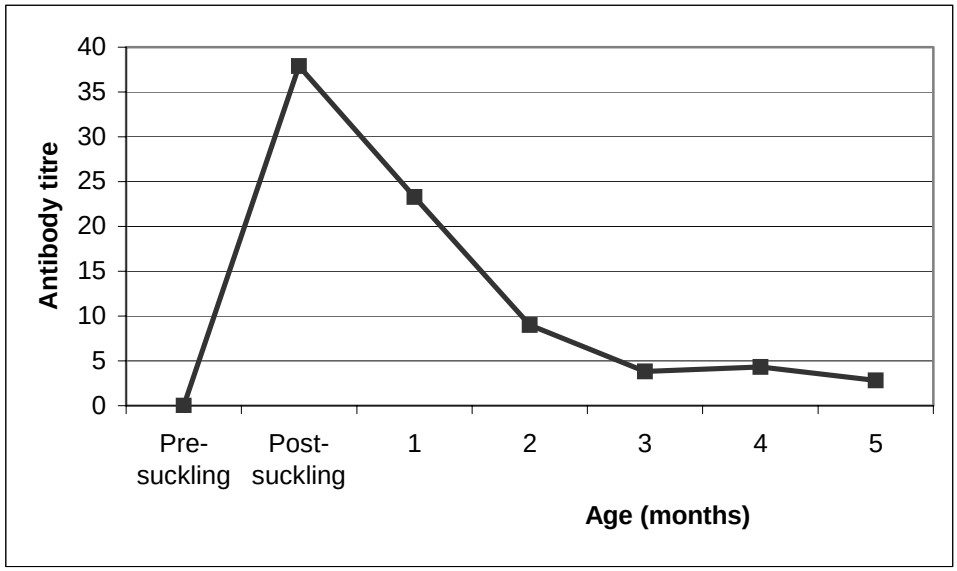

Figure 20: Mean CF colostrum-derived serum antibody titres to EHV-4 in the 30 foals.

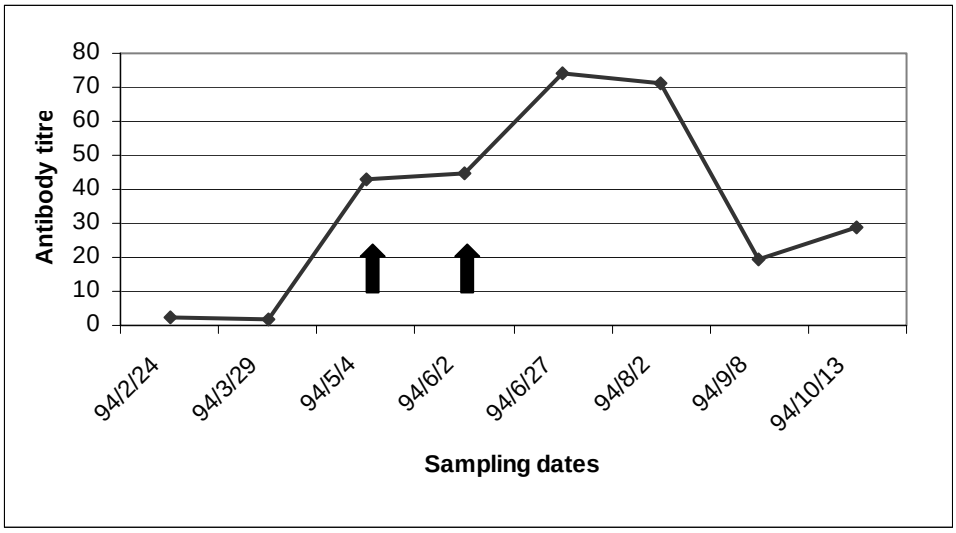

Figure 21: Mean serum CF antibodies to EHV-4 in the 30 foals (The black arrows indicate when foals were vaccinated with Fluvac EHV4/1 Plus).

Mares with antibodies to ERV-1 transferred them to their foals (Table 18 in Appendix V and Figure 22). The post-colostrum antibody titres ranged from 0 to 1:320. Colostrum-derived antibodies to ERV-1 were immeasurable in 4 of the foals (F13, F26, F30 and F35). By 2, 3, 4, 5 and 6 months of age, respectively, one, 12, seven, four and three foals had no more antibodies. No further antibodies were detected in the foals (Table 11).

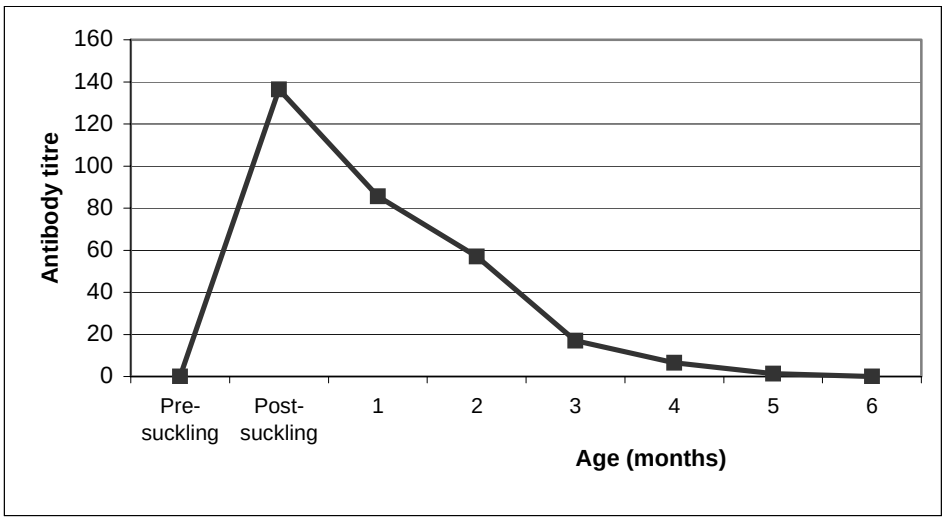

Figure 22: Mean SN maternally-derived serum antibodies to ERV-1 in the 30 foals.

In the case of ERV-2 maternal immunity waned after the foals were 1 to 5 months of age (Figure 23). The post-colostrum neutralizing antibody titres ranged from 0 to 1:80. Colostrum-derived antibodies to ERV-2 were immeasurable in nine of the foals (F8, F13, F20, F22 - F24, F26, F30 and F35). In six, five, five, two and one foals the colostrum-derived neutralizing antibody titres had waned by the time the foals were, respectively, 1, 2, 3, 4 and 5 months of age (Table 19 in Appendix V).

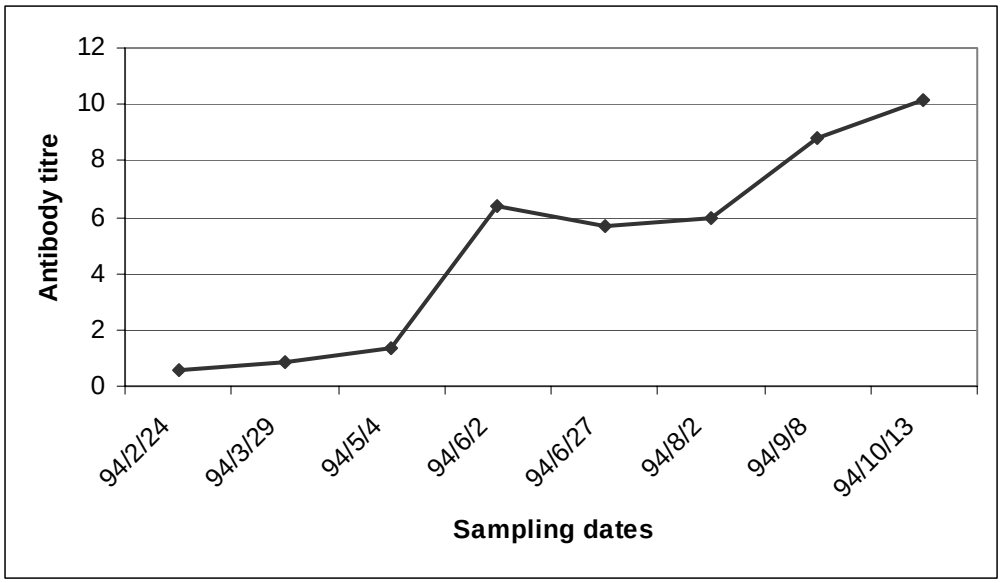

Figure 23: Mean SN colostrum-derived serum antibody titres to ERV-2 in the 30 foals.

There was a slow spread of this virus through the population with only two foals with detectable antibodies on 4/05/1994 sampling until 18 of the 30 foals (60 %) had antibodies at the end of the sampling period (Figure 24). In F6 and F13 a small rise in antibody titre was noted at 3 months of age. In two of the foals (F6 and F33) a rise in antibody titre occurred on two occasions.

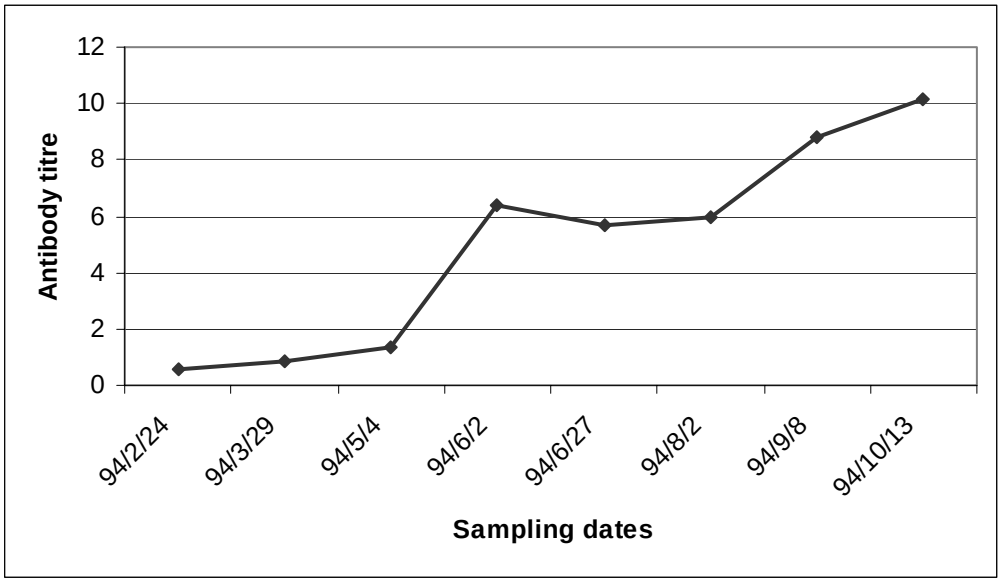

Figure 24: Mean serum SN antibody titre to ERV-2 in the 30 foals.

As in the case of the other viruses tested, the colostrum-derived immunity to EAdV-1 in the foals had waned by the age of 6 months (Figure 25). The post-colostrum neutralizing antibody titres ranged from 0 to 1:320. Colostrum-derived antibodies to EAdV-1 were immeasurable in F26. Although no post-colostrum antibodies were detected in F24, the subsequent monthly samplings indicate that they were present. In three, seven, nine, five, two and one foals the colostrum-derived neutralizing antibody titres had waned by the time the foals were, respectively, 2, 3, 4, 5 and 6 months of age.

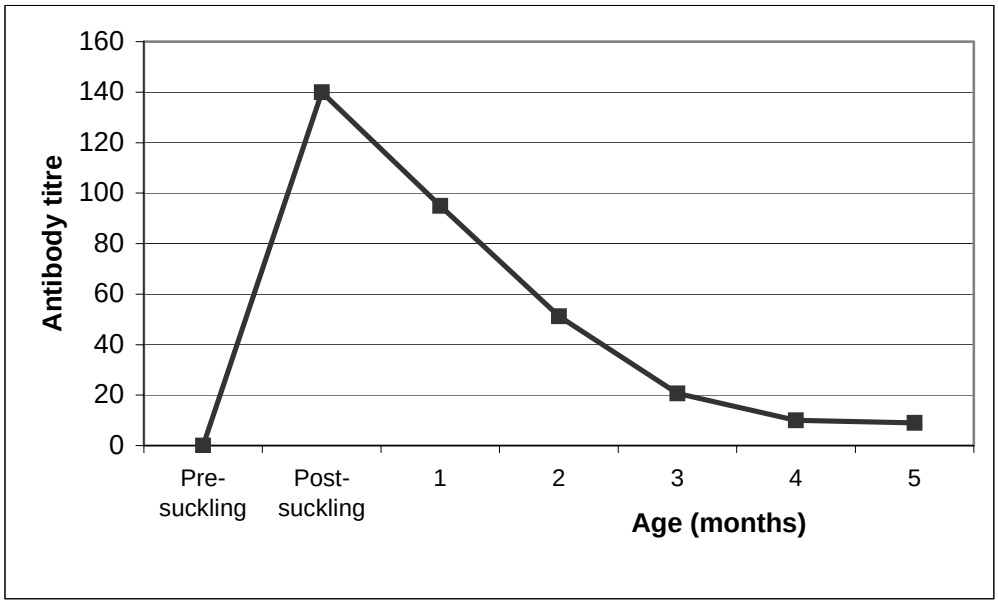

Figure 25: Mean SN colostrum-derived serum antibody titres to EAdV-1 in the 30 foals.

Natural infection occurred with the virus when the foals were 5 to 6 months of age and spread slowly among the foals with the result that by the end of the sampling period 23 of the 30 foals (76,67 %) had antibody titres to the virus (Figure 26). Eleven foals (36,67 %) had two or more increases in antibody titre by the time they were one year of age. At the last sampling there was a marked rise in the mean antibody titre in these horses with eight of them (26,67 %) showing a 4-fold rise in antibody titre (Table 20 in Appendix V and Figure 26).

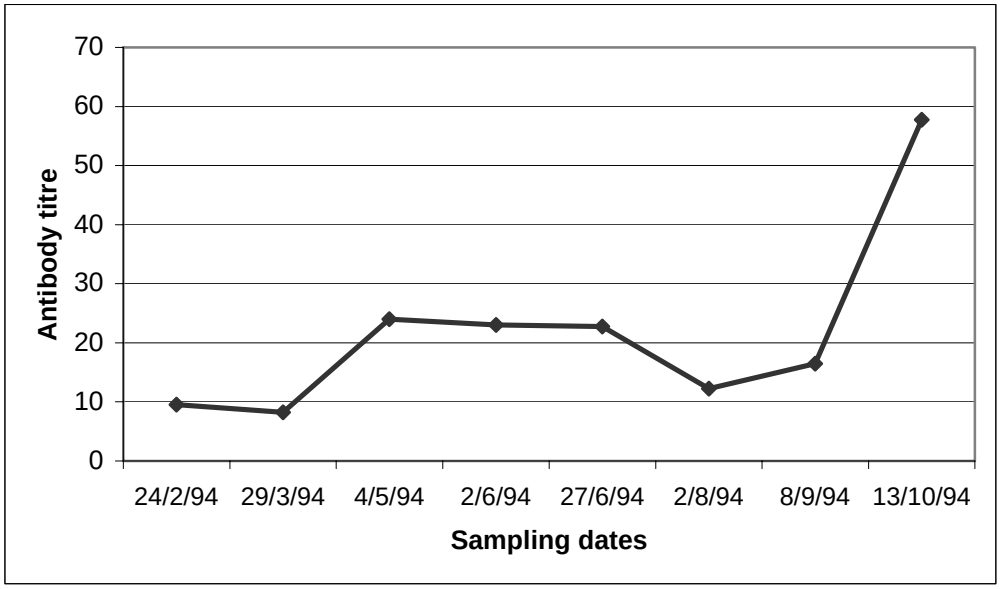

Figure 26: Mean serum SN antibody titres to EAdV-1 in the 30 foals.

Mean HI colostrum-derived antibodies to EIV had waned to low levels by the time the foals were 3 to 7 months of age (Figure 27). The post-colostrum neutralizing antibody titres ranged from 0 to 1:1280. Colostrum-derived antibodies to EIV were immeasurable in F26. In one, two, four, six, thirteen and four foals the colostrum-derived neutralizing antibody titres had waned by the time the foals were, respectively, 2, 4, 5, 6, 7 and 8 months of age.

A rise in HI antibody titres to the virus was noted after vaccination with Fluvac EHV 4/1 Plus, as indicated by the black arrows in Figure 28, the greater rise being after the booster vaccination was administered. Thereafter an erratic pattern of drop and rise in antibody titres was noted.

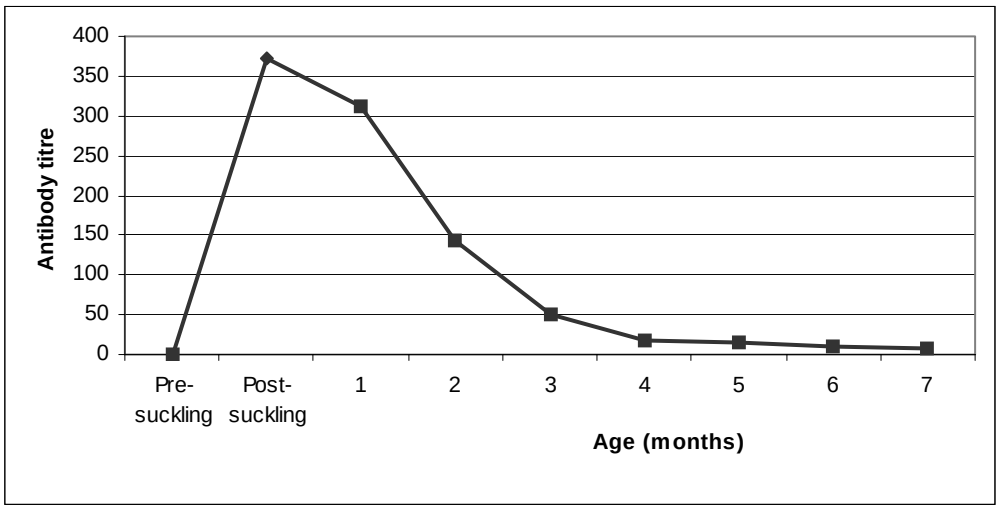

Figure 27: Mean HI colostrum-derived serum antibody titres to EIV in the 30 foals.

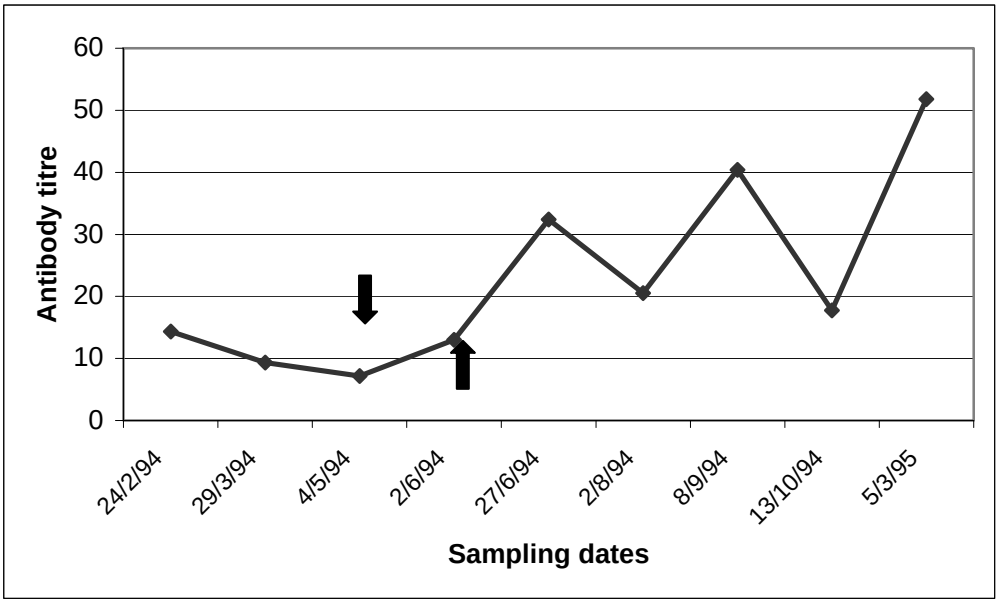

Figure 28: Mean serum HI antibody titres to EIV in the 30 foals (The black arrows indicate when foals were vaccinated with Fluvac EHV4/1).

CHAPTER FOUR: DISCUSSION AND CONCLUSIONS

4.1 DISCUSSION

4.1.1 METHODS USED

For practical purposes specimens were collected only on a monthly basis. As a result not all viruses circulating within the population could be isolated or detected since the duration of shedding of respiratory viruses is short. In the case of EIV, EHV-1 and EHV-4, it is usually not longer than 10 days after initial infection and 24 to 48 hours on subsequent infections (Mumford, 1994b; Mumford & Rossdale, 1980). Furthermore, viral shedding may begin before clinical signs of respiratory tract disease occur. Often clinical signs are only noted if they are severe or when there are secondary bacterial infections. These are the possible explanations why only EHV-4 was cultured from nasopharyngeal swabs and not any of the other viruses that were circulating in the population. The serological results indicate that EAdV and ERV-2 were also circulating in these foals during the course of the investigation.

The sampling interval used is suitable, however, for monitoring the serological response of animals to viral infections. In this way it can be determined when foals are infected for the first time, how a viral disease spreads through a population and when they are re-infected. Such regular sampling of a group of horses for serology (i.e. monthly), gives a more accurate picture of the infectious agent(s) that are involved than the examination of serum specimens taken only during the acute stage and then 2 to 3 weeks later. In the latter instance, the time of sampling is critical in order to obtain a 4-fold rise in antibody levels. It is, however, impractical since the clinical signs are not always noted in the acute stage of respiratory infections. The time to reach maximal antibody titres varies in different viral infections (Chambers *et al*., 1994a; Mumford & Rossdale, 1980).

Despite the fact that there are newer techniques, such as antigen-capture ELISAs and various molecular-based methods, available for the detection of certain viral respiratory diseases such as EI, EHV-1 and EHV-4 infections (Cook *et al*., 1988; Livesay *et al*., 1993; Oxburgh & Hagstrom, 1999; Yeargan *et al*., 1985), it was decided to use the traditional method of tissue culturing to isolate and identify respiratory viruses. This is the method most suited for monitoring a normal foal population having more than one respiratory virus circulating during a prolonged investigation. The use of EEL, RK13 and Vero tissue cultures are suitable for the replication of all respiratory viruses except EIV. This is not the case when using newer techniques as they are virus-specific. These tests are also very expensive particularly if a wide range of viruses is to be tested. Although tissue culturing is less

sensitive than many of the other methods as it requires large numbers of viable infectious viral particles, all efforts were made to reduce this problem by proper sampling, transport and storage of the specimens and by the use of high quality tissue cultures. The specimens were also passaged three times at weekly intervals onto tissue culture to increase the number of viral particles.

It was decided to culture for bacteria and fungi using general laboratory media either aerobically or in an increased atmosphere of carbon dioxide as all the known bacterial and fungal pathogens of the upper respiratory tract will grow under these conditions. Anaerobic bacterial culturing would only have been done in the case of suspected lower respiratory tract infections (Hoffman *et al.,* 1993b; Sweeney *et al.,* 1985). No lower respiratory tract disease was detected by clinical examination in this study, although a foal, not in the study group, died at 3 months of age due to pleuropneumonia caused by *S. equi* subsp. *zooepidemicus*. A wide variety of culture media is usually required to grow mycoplasmas. However, only Hayflick's and Chalquest media were selected as they are known to support the growth of mycoplasmas recognized as pathogenic in horses.

It was decided to identify only those bacteria that have in the literature been regarded as potential pathogens to species level to reduce the costs and time required to do so. The sampling for the bacteria, including mycoplasmas and fungi was also terminated 2 months earlier than the other samplings for the abovementioned reasons.

4.1.2 NORMAL MICROFLORA OF THE UPPER RESPIRATORY TRACT

It is these bacteria that cause infections of both the upper and lower respiratory tracts when the protective barriers and immune system are compromised. In the healthy horse, with the exception of *S. equi* subsp. *equi*, *C. neoformans* and *B. mallei*, all bacteria and fungi isolated from the upper respiratory tract are usually present either as commensal or transient microflora. Bacterial commensals in the nasopharynx serve as a source of pathogens in the immune-compromised or damaged upper and lower respiratory tract (Bailey & Love, 1991; Smith & Robinson, 1981). Thus it is important not only to determine what bacteria routinely colonize this area and whether their composition changes with age, locality or infection with, for example, viruses, but also to distinguish between the transitory and commensal microflora (Martens *et al*., 1981).

A large variety of bacterial species were cultured from the nasopharynx of the foals throughout the sampling period. Although not speciated, it appears that the most predominant and usually non-pathogenic coagulase-negative staphylococci, viridans streptococci, *Moraxella* spp. and *Flavobacterium* spp. were commensals of the nasopharynx of these foals. During this study it became apparent why *A. equuli* is a common cause of neonatal septicaemia in foals with insufficient passive immunity (Robinson *et al*., 1993) as many foals (33,3 %) carried it in their nasopharynx during the suckling period. It seems to be

one the first bacteria to colonize the nasopharynx and gradually becomes less common as the foal grows older. This implies that it is part of the permanent microflora of the nasopharynx of suckling foals but not necessarily in the older foals, as none were isolated in the last two samplings. It does appear that *A. equuli* is not commonly isolated from the nasopharynx in older horses but rather from the oral cavity (Hoquet *et al*., 1985; Platt, 1973; Sternberg, 1998). It is believed that *A. equuli* causing septicaemia in foals originates from the oral cavity of mares (Sternberg, 1998).

Streptococcus equi subsp. *zooepidemicus* is the most common potential pathogen in horses and is regarded as being part of the commensal microflora of the nasopharynx and tonsils (Kamada & Akiyama, 1975; Welsh, 1984; Woolcock, 1975). This was substantiated in this study as it colonized the nasopharynx of some foals from one month of age and gradually colonized more foals, and although it was not isolated at every sampling, it established itself in eight foals.

Staphylococcus aureus was a common isolate from foals throughout the study period, but the numbers of infected foals were greatest in the first 2 months after weaning. *Staphylococcus aureus* is more commonly associated with infections of the skin, eye, genital tract, joints and bones. Although this bacterium has been associated with upper respiratory tract disease especially those initiated by viral infections such as EIV (Sarasola *et al*., 1992; Shimizu *et al*., 1991), it should rather be considered as part of the commensal nasopharyngeal microflora. Hoffman *et al*. (1993a) found that this bacterium, when isolated from bronchial exudate, has no correlation with distal airway disease.

It was not possible in this study to determine if the mycoplasmal isolates contained among them potential pathogens such as *M. felis* which causes pleuropneumonia in horses (Hoffman *et al.*, 1992; Ogilive *et al.*, 1983; Rosendal *et al.,* 1986; Wood *et al*., 1997). However, there was no clinical evidence of lower respiratory tract infection in these foals and mycoplasmas were cultured from most specimens, irrespective of whether the foals were clinically normal or ill. This implies that these *Mycoplasma* species are commensals of the upper respiratory tract and did not play a role in respiratory disease of the foals in this study.

The fact that the number of fungal species isolated from the foals increased with age is possibly because young foals are suckling and are less likely to eat hay that is often contaminated with fungal spores that are easily inhaled. There was no increase in the number of species of fungi isolated during the summer months of October to March as compared with the samplings during the winter months from April to August. This is contrary to what is usually found, as warm and wet weather increases the fungal load in organic material (Clarke, 1987a). Reasons for this finding could be that the number of fungi found in the nasopharynx were not quantified, fungal spores during wet weather are less able to become air-borne and that the foals were not stabled in the summer months. A greater

variety of fungal species was cultured from the nasopharynx of the foals that were stabled in winter. This is indicative of greater exposure to fungal spores in the bedding of the stables (Clarke, 1987a). It also appears that fungi isolated are transient microflora as they were not consistently isolated from consecutive samples from the same horse.

4.1.3 INFECTIONS FROM BIRTH UNTIL 5 MONTHS OF AGE

Five of the foals had virus-specific antibodies to EHV-1, EHV-4, ERV-1, ERV-2 and EAdV in their pre-suckling serum, possibly because the foals had suckled unbeknown to the person who did the sampling. Another reason could have been in response to an *in utero* infection (Hammer *et al*., 2001; Sheoran *et al*., 2000). As there was no evidence of viral infection in these foals in the neonatal period and the fact that the antibody levels rose in the post-suckling sera, it was concluded that the antibody titres present were the consequence of suckling and these findings were excluded from the results. Foals F13 and F26 often had elevated rectal temperatures in the suckling period and more often suffered from upper respiratory tract infections during this period than did the other foals. Although these foals lacked antibodies to the viruses tested, it was not established whether or not they had low levels of gammaglobulins in their sera.

The virus-specific maternally-derived antibodies in foals 1 day after birth were generally higher than those of the mares prior to partus. This is in agreement with previous findings that the IgGa and IgGb subclasses were up to four times higher in colostrum on the day of partus and slightly higher in the serum of 1-day-old foals than those in the mares' (McGuire & Crawford, 1973; Sheoran *et al*., 2000). It has, however, been reported that EHV-1 and EHV-4 titres were similar in foals' sera than those of their dams (McGuire & Crawford, 1973). In this study the mean colostrum-derived serum SN antibodies levels to EHV-1 and SN and CF antibody levels to EHV-4 but not CF antibody levels to EHV-1 were equal or lower than those of the mares' mean serum antibody levels.

In individual foals in this study, the duration of measurable colostrum-derived serum antibodies to all the viruses varied from 2 to 7 months after birth and dropped at a rate directly proportional to the initial antibody titre. These results correlate with other published findings (McGuire & Crawford, 1973). Sheoran *et al.* (2000) found that IgGa, IgGb and IgG(T) were depleted in foals by days 28, 63 and 42 respectively. Equid herpesvirus-1 and EHV-4 antibody titres are usually depleted by the time foals are 100 days of age (Bagust *et al*., 1972). Of interest is that even after maternal immunity had waned, most of the foals did not become infected with respiratory viruses until 5 to 6 months of age. This is similar to the findings of Allen (2002), who reported that foals with maternally-derived antibodies to herpesviruses tend to become infected from 4 to 12 months of age.

The elevated body temperature in the foals sampled in September and October 1993 was most probably due to fact that a body temperature of 39 °C is considered normal for neonatal

foals during the warmer spring and early summer months. From the ages of 2 to 5 months, it was at times difficult to capture and handle the foals. During summer (October to February) the daily day ambient temperature was high, being on average 26 °C in the shade, and reaching 36 °C on some days. Sampling started at 0800 and usually continued until 1300. Foals that were sampled early generally had lower body temperatures than those sampled later in the day. Thus at the time of sampling, the heart and respiratory rates and the body temperature of some of the foals were elevated due to excitement and high ambient temperatures, making these parameters difficult to assess. This effect is clearly observed in Figure 9, where many foals, during the 23/11/1993 to 4/05/1994 sampling period, appeared healthy but had an elevated rectal temperature. Since the rectal temperatures were not taken daily of each foal, it was also difficult to have a knowledge of each foals temperature pattern.

The presence of a serous nasal discharge which may be considered to be an indicator of a viral infection of the respiratory tract was also difficult to assess because a dusty environment may also give rise to similar clinical signs in foals (Traub-Dargatz, 1997). In most of the foals there was no evidence of viral infection prior to the 29/03/1994 sampling period. The exceptions were foals F6 and F13 which were infected at 3 months of age with ERV-2, and foals F3 and F34 which were infected with EAdV at 4 months of age. These four foals at that time also had clinical signs of upper respiratory disease. Other than foal F13, which did not have any measurable virus-specific, colostrum-derived antibodies, it is not known why they were infected earlier than the other foals. It is likely, however, that they were in close contact with mares that were shedding high numbers of virus at that time. This was not tested.

During the suckling period many foals exhibited a mucoid to purulent nasal discharge, which was accompanied, with the exception of a few foals, by a fever reaction. Apart from the abovementioned foals no viruses or serological evidence of a viral infection was detected in these foals. However, several opportunistic bacterial pathogens of the respiratory tract were isolated. During the 26/10/1993 sampling a pure culture of numerous *A. equuli* colonies was cultured from the nasopharynx of foal F29 and a mixed culture from four other foals (F11, F23, F34 and F35). These five foals had clinical signs of upper respiratory tract disease. In later samplings *A. equuli* was equally isolated from foals with clinical signs of respiratory tract disease and healthy foals. On 23/11/1994, *S. equi* subsp. *zooepidemicus* was isolated from the nasopharynx of nine of the 11 foals with evidence of upper respiratory tract disease, which is indicative that it may be the cause of pharyngitis in these foals.

4.1.4 INFECTIONS FROM FIVE MONTHS TO ONE YEAR OF AGE

The number of foals with upper respiratory infections increased when they were 5 to 6 months of age and continued until they were 9 months of age and, in some foals, up to 12 months of age. The earlier period coincides with the period of waning maternally-acquired

immunity when foals are especially susceptible to viral infections of the respiratory tract which usually require veterinary intervention (Cohen, 1994; Studdert, 1971).

On 29/03/1994 EHV-4 was isolated from nasopharyngeal swabs of eight (26,7 %) of the foals, which is a figure higher than that obtained in studies done in England (Burrows *et al*., 1982; Mumford *et al*., 1998). The main reason why this was the case is possibly that foals, infected for the first time, usually shed more virus and for a longer period of time, making its detection easier. Although molecular methods were not used in the identification of these viruses, it was considered that they were all EHV-4 as they were identified as a herpesvirus on TEM, grew on EEL cells but not RK13 cells and were better neutralized by antibodies generated to EHV-4. The fact that no viruses were cultured from the heparinized blood specimens further supports evidence that the virus circulating in the population when the viruses were isolated was EHV-4 and not EHV-1 as it tends to be limited to cells of the upper respiratory tract and rarely causes a viraemia (Edington & Bridges, 1990).

The source of EHV-4 was most probably the mares since horses, once infected, can be latent carriers (Welch *et al.*, 1992) or they can be repeatedly infected, periodically shedding virus in their respiratory tract secretions (Thomson, 1978). One of the foals (F3) from which EHV-4 was cultured had a fever when sampled and 6 manifested either a purulent nasal discharge or swelling of the lymph nodes of the head. Ten of the foals from which no virus was isolated had a rectal temperature of 39 °C or higher, or presented with a purulent nasal discharge and swollen mandibular lymph nodes.

During the abovementioned sampling period a number of potential bacterial pathogens were cultured from the nasopharynx of some of the foals, namely, *S. equi* subsp. *zooepidemicus*, *S. aureus*, A*. equuli* and *A. lignieresii*. However, only *S. equi* subsp. *zooepidemicus* was consistently associated with respiratory tract disease.

Since the foals were infected 2 to 6 weeks prior to weaning, they only again become infected by EHV-4 when immunity waned and not at or just after weaning. In Australia, Gilkerson *et al*. (1994) found that most of the EHV-4 shedding did occur at weaning and during March. Like Gilkerson's study, EHV-4 was also isolated in March, but the foals in the Australian study were weaned a month earlier than those in this study.

Of interest is the increase in number of foals that did not have a fever reaction after weaning but showed clinical signs of upper respiratory disease (Figure 9). This occurs either when previously infected foals are re-infected or when only the upper respiratory tract is affected (Traub-Dargatz, 1997). Especially after weaning respiratory infections when they occurred tended to spread rapidly within a camp, but they were more common in the stabled weanlings. Spread of respiratory disease was slow between neighbouring camps. This is indicative that nasal contact was most probably the more important means of transmission. In the 02/06/1994 sampling period EHV-4 was only cultured from one foal (F3), although 22

of the 30 foals, including F3, had clinical signs of upper respiratory tract disease. Fifteen of the foals had a rise in SN antibody titre in serum taken nearly a month later (Table 16 in Appendix V). Thus there seems to be a relatively good correlation between shedding of virus and clinical signs of respiratory disease. However, it was not possible to time the swab taking with maximum virus shedding, thus many more foals were had increase in antibody titre and were sick than were those from which EHV-4 was cultured. This observation is similar to that of Doll & Bryans (1963). Reasons for this observation could be that the foals had recovered from the fever reaction but were still shedding virus, and that those foals with a fever and/or clinical signs of upper respiratory disease may either have stopped shedding virus and/or have been suffering from secondary bacterial infections (Mumford & Rossdale, 1980). The fact that EHV-4 was again isolated from foal F3 two months later indicates that immunity to this virus is short-lived. This is in agreement with Thomson (1978), who found that protective immunity to EHV-4 can be in some foals as short as 2 months.

During this sampling period the most common opportunistic bacterial pathogen was *S. equi* subsp. *zooepidemicus*. It was isolated from both the nasopharynx and/or lymph nodes of the head of 50 % of the foals. In fact, from the sampling on 29/03/1994 and for the rest of the sampling period this bacterium was the predominant one isolated from foals with respiratory tract disease. This finding is similar to other reports that implicate *S. equi* subsp. *zooepidemicus* as the most common bacterial infection of the respiratory tract of 3- to 8-month-old foals (Hoffman *et al.*, 1999; Lavoie *et al*., 1991; Timoney, 1993; Timoney *et al*., 1994). The agent of strangles, *S. equi* subsp. *equi* was not cultured during this study. At that time there was also no evidence that the bacterium was present on the farm or even in South Africa.

Although EHV-1 was circulating in some of the mares, eight mares aborted 2 months after their foals were weaned as a result of EHV-1 infection, at the time when EHV-4 was isolated from the foals, it was not isolated from the foals. This supports the observations of researchers in Kentucky and Japan that foals most probably do not play a major role in the epidemiology of EHV-1 abortions in mares (Allen & Bryans, 1986; Matsumura, *et al*., 1992).

In this study, the rise in SN and CF antibody titres to EHV-1 and EHV-4 almost were similar, with the exception that EHV-4 antibody titres rose at an earlier age and were slightly higher than those to EHV-1. The SN antibodies to EHV-4 rose sharply after infection with EHV-4 but the SN antibodies to EHV-1 only rose after the foals had been vaccinated with Fluvac EHV-4/1 Plus. The slight rise in SN antibody titres to EHV-1 prior to vaccination may have been the result of cross-reaction with EHV-4 or non-specific cross-reactions that occasionally occur when the measured antibody titres are low. Clabough (1991) has recorded that SN antibodies to EHV-1 tend to be virus-specific whereas those to EHV-4 elicit cross-reactive antibodies to EHV-1. Edington (1990) showed that experimental infection of foals either with EHV-4 or EHV-1

resulted in a greater rise in antibody titre of the homologous virus and a smaller rise in the antibody tire of the heterologous virus. The initial rise in CF antibody titres was greater to EHV-4 than to EHV-1, indicating that the early rise in SN antibody titres was due to cross-reactive antibodies to EHV-4. These results support the virological results that showed that EHV-4 was circulating in the foals just prior to weaning. The CF titres to EHV-1 and EHV-4 developed earlier and waned more rapidly than the SN antibody titres. Similar findings have been recorded by several authors who consider CF antibody titres to be more reliable than SN antibody titres for the diagnosis of recent infections (Doll & Bryans, 1962; Mumford & Bates, 1984). Doll & Bryans (1962) and Edington & Bridges (1990) found that the primary CF antibody response to EHV-1 occurred between days 8 and 10 post-infection with maximum titres being reached between days 14 to 21. Titres receded between 30 and 60 days. Serum neutralizing antibodies were detected between days 9 and 12, maximum titres being reached between days 14 and 21. A complicating fact in the interpretation of these results was the vaccination of the foals with Fluvac EHV 4/1 Plus just after they had been exposed to EHV-4. Thus the rise in titres after vaccination appears to be those expected of a vaccine response. The SN antibody titre rise to EHV-1 after vaccination was poor; this finding correlates with a published report that vaccination usually elicits a weak SN antibody response in younger horses, with the response being stronger in horses that have previously been exposed to vaccine challenge (Mumford & Bates, 1984).

The foals in this study had no serological evidence that they had been infected with ERV-1 in their first year of life. This is in agreement with previously published reports (Hofer *et al.*, 1973; Li *et al.*, 1997), in which it was noted that ERV-2 but not ERV-1 infects foals in their first year of life. Post-weaning, the mean antibody titres in the foals to ERV-2 were low mainly because the infection spread slowly with only a few foals per sampling period having antibody titres of greater than 1:10. It does appear that ERV-2 did not cause noticeable respiratory disease in foals with antibodies to the virus, as the foals that had evidence of respiratory disease also had elevated antibody tires to either EHV-4 or EAdV.

Even though it was not cultured, EAdV-1 appears to have been circulating in the foal population. Serological results indicated that all of the foals had been infected at least once and some even three times in their first year of life. It was difficult to determine what role this virus played in respiratory disease as the rise in antibody titre occurred during the same periods when EHV-4 circulated in the foal population. However, during the last sampling period on 13/10/1994 there was a four-fold rise in antibodies of six foals without marked rise in antibodies to the other viruses. Of those foals only three had clinical signs of respiratory disease and only one with evidence of secondary bacterial infection due to *S. equi* subsp. *zooepidemicus*. Despite the fact that EAdV-1 infected all the foals it appeared to be much less important than EHV-4 in causing respiratory tract disease. This is in agreement with other reports which indicate that EAdV-1 only causes mild respiratory disease in immune-

competent foals but can cause fatal disease in Arabian foals suffering from primary severe combined immunodeficiency disease (Studdert, 2003).

When this study was done, EIV had not been identified in South Africa since the 1986 outbreak. Therefore, no attempt was made to culture it. It was, however, included in the serological investigation, using the HIT. The results obtained reveal there was a good level of maternally-derived immunity in these foals. This test was able at that time to identify all H3N8 strains as it contained A/equine 2/Newmarket/79, which was the virus that was isolated in South Africa during the 1986 equine influenza outbreak (Bogdan *et al*., 1992; Hannant *et al*., 1988; Rogers, 1988). The colostrum-derived antibodies were much higher than those attained in the serum of the foals post-vaccination because the mares had high antibody titres as they had been vaccinated within one month of the birth of their foals and had been vaccinated twice a year since they were born. The older mares (more than 7 years of age) were naturally infected during the 1986 outbreak.

It was considered that the initial rise in antibody to EIV was due to vaccination of the foals with an anmestic response being noted only after the booster vaccine had been administered (see Figure 28). Although no culturing was done for the presence of EIV, the antibody titres after the initial rise were erratic and there was no clear pattern of elevation of titres in individual foals. Therefore it was concluded that there was no evidence that the EIV that was isolated in the 1986 outbreak was circulating in this population at the time of the study. Antibody titres when using the HIT tend to be very variable and are affected by how the virus was propagated, the diluent used in the test, and the source and age of chicken red blood cells. For example, EIV grown in primary monkey kidney cells gives a two- to four-fold higher antibody titre to virus cultured in 9-day-old embryonated hen's eggs and PBS when used as a diluent will give higher titres than allantoic fluid of bovine serum (Bogdan, *et al*., 1992). One of the reasons for the erratic results could be that, although batch testing was done and the test was repeated four times on different days, not all the samples could be handled on the same day and thus the tests had to be done on different days. Mumford *et al*., (1998) reported that under such circumstances, even though the same chicken blood is used and identical test conditions prevail, variability can still occur.

4.2 CONCLUSIONS

This study of respiratory tract disease in a group of foals on a Thoroughbred breeding establishment showed that foals that received adequate maternally-derived immunity usually suffered from repeated viral infections of the respiratory tract from 5 months until 12 months of age. This is in agreement with studies on horse breeding farms worldwide (Gilkerson *et al*., 1994; Thomson, 1978). The most clinically significant virus was EHV-4: as in primary infection it induced both an elevated rectal temperature and clinical signs of respiratory disease, while during subsequent infections only clinical evidence of upper respiratory tract

infections was present. Equine adenovirus-1 played a lesser role in respiratory infections in the foals while ERV-2 appeared not have infected as many foals as EHV-4 did, and it did not cause respiratory tract disease.

Before 5 months of age, only a few foals had clinical evidence of respiratory disease which could possibly either be attributed to other respiratory viruses not tested i.e. EHV-2 or to bacterial infections resulting from the irritation of the nasal mucosae by dust. Weaning stress as well as infection with EHV-4 led to secondary infection with primarily *S. equi* subsp. *zooepidemicus* resulting in more severe clinical signs of upper respiratory tract disease.

Foals may shed large numbers of EHV-1 virus particles from their respiratory tracts and for a longer period than adults and therefore they act as amplifiers of this virus and thus sources of infection for the mares (Doll & Bryans, 1962) However, on farms that manage their weaned foals separately from the pregnant mares it is unlikely that foals act as amplifiers of infection (Bryans, 1981). This was the case on the farm in the investigation as there was no evidence of EHV-1 infection in the foals in their first year of life, notwithstanding the fact that an outbreak of EHV-1 infection was diagnosed in the mares.

Most of the bacteria and fungi isolated from the nasopharynx are transient, entering it by either inhalation or ingestion. Bacteria that established themselves on the nasopharynx of these foals were coagulase-negative staphylococci, viridans streptococci, mycoplasmas and *Moraxella* spp.. *Actinobacillus equuli* was a commensal in foals up to 3 months of age and *S. equi* subsp. zooepidemicus and *S. aureus* throughout the first year of life.

ADDENDICES

APPENDIX I: REAGENTS AND MEDIA

ALSEVERS SOLUTION

Glucose anhydrous ($C_6H_{12}O_6$)[1]	42,0 g
Sodium chloride (NaCl)[1]	8,4 g
Sodium citrate ($Na_3C_6H_5O_7.2H_2O$)[1]	16,0 g
Citric acid ($(CH_2COOH)_2.H_2O$)[1]	1,10 g
De-ionised water	2000 ml

METHOD

1. Dissolve the chemicals in the listed order in water.
2. Bottle in 50 ml volumes and sterilize by autoclaving at 110 °C for 10 minutes.
3. Store at 4 °C until required.

CALCIUM- AND MAGNESIUM-FREE PHOSPHATE BUFFERED SALINE (PBS-)

Sodium chloride (NaCl)[1]	80,0 g
Potassium chloride (KCl)[1]	2,0 g
Disodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$)[1]	11,05 g
Potassium orthophosphate (KH_2PO_4)[1]	2,0 g
Dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$)[1]	2,0 g
Twice deionised water	1000 ml
Phenol red 1 % solution	10 ml

METHOD

1. Dissolve the salts into the water in the order listed and aliquot into 100ml volumes.
2. Sterilize by autoclaving at 121°C for 20 minutes.
3. Store at room temperature (22°C) until required

PHOSPHATE BASIC SOLUTION OF DULBECCO (PBS+)

SOLUTION A

Sodium chloride (NaCl)[1]	80,0 g
Potassium chloride (KCl)[1]	2,0 g
Disodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$)[1]	14,4 g
Potassium orthophosphate (KH_2PO_4)[1]	2,0 g
Twice deionised water	500 ml
Phenol Red (1 % solution)[1]	5 ml

SOLUTION B

Calcium chloride ($CaCl_2$)[1]	0,5 g
Twice deionised water	250 ml

SOLUTION C

Magnesium chloride heaxahydrate ($MgCl_2.6H_2O$)[1]	0,5 g
Twice deionised water	250 ml

METHOD

1. Make solutions A, B and C separately by dissolving the salts in the water as listed.
2. Dispense Solution A in 50 ml volumes and Solutions B and C in 25 ml volumes in glass containers.
3. Sterilize the solution by autoclaving at 121 °C for 20 minutes.
4. Store at room temperature (22 °C) until required.
5. Just before use add 50 ml of solution A and 25 ml of solutions B and C to 900 ml of sterile twice de-ionised water.

CRYOPERSERVATION OF VIRUS

The viruses 0,8 ml of virus was placed in a freezing solution of 10 % foetal calf serum (FCS) in buffered lactose peptone (BLP) with 1 ml each of penicillin and streptomycin added to 100 ml of BLP.

PREPARATION OF BUFFERED LACTOSE PEPTONE (BLP)

1. Dissolve 18,1 g of disodium hydrogen orthophosphate (Na_2HPO_4)[1] and 2,64 g of potassium orthophosphate (KH_2PO_4)[1] in 3 000 ml of de-ionised water.
2. Dissolve 60 g of peptone (Difco) and 300 g of lactose into this solution.
3. Adjust the pH to 7.4.
4. Sterilized by filtration through a 22 μm filter (Millipore, SA)
5. Check for sterility by plating out onto bacteriological solid media and incubate for 72 hours.
6. Bottle in 50 ml volumes and store at 4 °C

CRYOPERSERVATION OF CELLS
STORAGE MEDIUM OF CELLS

Eagles-minimum essential medium (E-MEM) (Highveld Biologicals, South Africa)	60 ml
Foetal calf serum (FCS) (Highveld Biological, South Africa)	30 ml
Dimethyl sulfoxide (DMSO)[1]	10 ml

METHOD

1. To sterility checked E-MEM and FCS add DMSO
7. Dispense in 10 ml volumes and store at –20 °C
2. Dependent on the split ratio of the cell line, 2 ml of storage medium is added to cell pellets with a split ratio of 1:3 and 3 ml to cell with a split ratio of 1:4.
3. Dispense 1 ml into each labelled cryotube.
8. Wrap in cotton wool, place upright and freeze at –70 °C.

TRYPSIN-VERSENE SOLUTION (ATV)

Versene (EDTA)	4 g
Sodium chloride (NaCl)[1]	160,0 g
Potassium chloride (KCl)[1]	8,0 g
Sodium hydrogen carbonate ($NaHCO_3$)[1]	11,6 g
Dextrose monohydrate ($C_6H_{12}O_6 \cdot H_2O$)[1]	10 g
Trypsin (Difco 1:250)	10 g
Phenol red (1% solution)[1]	10 ml
Twice deionised water	250 ml

METHOD

1. Dissolve the Versene in 300 ml of water.
2. Dissolve the salts and dextrose in 1000ml of water and mix with the Versene solution.
3. Add the trypsin powder, seal and incubate at 37 °C, shaking occasionally until the solution is clear.
4. Make up the solution to 2000ml with water and add phenol red.
5. Sterilize by filtering through a 0,22 μm filter (Millipore, SA)
6. Aliquot into 20 ml volumes and store at 4 °C
7. Check for sterility by culturing a small volume on a bacteriological agar plate at 37 °C for 72 hours.
8. Just before use dilute I part of ATV in 4 parts of sterile water. This solution will then be used to remove cells from the culture flasks.

VERONAL BUFFER (VBS) (pH 7.4 – 7.6)

STOCK SOLUTION

Sodium chloride (NaCl)[1]	83,80 g
Sodium bicarbonate ($NaHCO_3$)[1]	2,52 g
Barbitone sodium (sodium barbital)[1]	3,0 g
5,5 diethylbarbituric acid (barbital)[1]	4,60 g
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)[1]	1,00 g
Calcium chloride ($CaCl_2$)[1]	0,151 g

METHOD

1. Dissolve the first three chemicals in 1000 ml of hot deionised water.
2. Dissolve the last 3 chemicals in 500 ml of hot deionized water.
3. Cool both solutions and add the smaller volume solution to the first solution and make up the solution to two litres.
4. Store this stock solution at 4 °C.
5. Just before use dilute the stock solution 1:6 with deionized water (e.g. 50 ml of stock VBS with 250 ml of water)
6. To test the VBS, add VBS to SRBC and centrifuge, there should be no haemolysis.

LACTOPHENOL COTTON BLUE MOUNT FOR FILAMENTOUS FUNGI

Lactophenol blue is used both as a mounting fluid and a stain. Lactic acid acts as a clearing agent and aids in the preservation of fungal structures, phenol acts as a killing agent, glycerol prevents drying and cotton blue gives colour to the structures.

Stain preparation

Lactic acid[1]	20 ml
Phenol crystals (concentrated phenol)[1]	20 g
Glycerol or glycerine	40 ml
Deionized water	20 ml
Cotton blue anhydrous[1]	0,05 g
or 1% aqueous solution	2 ml

Dissolve phenol in the lactic acid, glycerol, and water by gently heating (if crystals were used). Then add cotton blue (Poirrier's blue and aniline blue are analogous to cotton blue). Mix well.

Staining procedure

1. Place a drop of the stain of a clean microscope slide.
2. Pick-up some fungal hyphae by either a dissecting needle or cellophane tape
3. Place in or on the drop of stain.
4. Place a coverslip on top and examine under a light microscope, with reduced condenser aperture.

Interpretation

Young fungal hyphae stain blue.

[2] All the listed chemicals originate from Sigma-Aldrich, South Africa

HAEMOCYTOMETER CELL COUNTING METHOD

1. Take 0,5 ml of cell suspension that has been collected after the cells were removed from a flask and fill one end of a counting chamber in haemocytometer using a 1 ml tuberculin syringe and 21 G needle.
2. Using a fresh sample fill the other end of the counting chamber.
3. Count at least four large squares containing 16 smaller squares on each chamber.
4. Take an average of the four counts.
5. The total number of cells will then be x 10^4.
6. The dilution factor is determined by the number of cells obtained divided by the number of required cells.

APPENDIX II: SEROLOGICAL METHODS

SERUM NEUTRALIZATION TEST

It was ensured that all the equipment, media and reagents used were free from micro-organism contaminants and that aseptic technique was used at all times, as contamination of the tissue cultures by bacteria (including mycoplasmas) and fungi would have negatively affected the results. It was also ensured that thoroughly washed and sterilized equipment was used that contained no cellular or chemical residues which could have been toxic to the cell cultures and/or caused their contamination. All the work was carried out in a laminar flow cabinet that had been regularly serviced.

Preparation of test sera

A 1:5 dilution of serum in PBS+ (refer to Appendix I) was made by adding 0,5 ml of prepared serum to 2 ml of PBS+ using a pipette. The diluted serum was then heated to 56 °C for 30 minutes, to inactivate non-specific viral inhibitors.

Preparation of cell cultures.

Stored cell cultures were rapidly defrosted at 37 °C and 1 ml was inoculated into 25 cm^2 tissue flasks containing 10 ml of cell culture medium with 10 % FCS. They were incubated with on their sides, with the caps slightly loose, in a 5 per cent CO_2 in air incubator until a confluent monolayer had formed. The cells in the flasks were collected and split according to the split ratio of that particular cell line. One of these flasks was used to continue the cell line and the others used in tests. The medium was poured off from the flasks and discarded. After washing twice using about 30 ml of PBS-, a further 20 ml of PBS- was added to each flask and placed in an incubator at 37 °C for 5 minutes (exception 15 to 20 minutes for Vero cells). After decanting the PBS-, 20 ml of ATV 1:5 diluted in water was added and gently spread over the entire surface of the flask by rocking the vessel side to side. After being in an incubator at 37 °C for approximately 3 minutes the flasks were checked to see whether the cells had detached from the wall and, if so, culture medium containing 10 % FCS was added to inactivate the trypsin. If they had not detached the flasks were incubated for up to 10 minutes longer. The contents of a flask was gently mixed by rotating the flask and were then poured into a 20 ml MacCartney bottle. Two millilitres of virus culture medium was added to the MacCartney bottle to further inactivate the trypsin. To sediment the cells the MacCartney bottle was centrifuged for 4 minutes at 650 g after which the supernatant was discarded and 10 ml of virus culture medium in 5 % FCS were added to the cell sediment. The mixture was agitated using a glass syringe and 15 G needle as previously described. The culture medium containing cells collected from a 150 cm^2 flask was made up 50 ml and the cells in the suspension were counted on a haemocytometer (refer to Appendix I). The cells were then

diluted in cell culture medium plus 5 % FCS to give the desired amount of cells per 80 µl. Dependent of the specific viral antibodies being determined the concentration of cells were the following: 4,8 x 10^5/80 µl of Vero and equine lung cells, and 4 x 10^5/80 µl of RK_{13} cells.

Preparation of test virus.

The viruses were propagated in susceptible tissue cultures. Equid herpesvirus 1, EHV-4, and EAdV were grown in primary equine lung cells, ERV-1 in Vero ATCC CCL81 (African green monkey) and ERV-2 in RK_{13} ATCC CCL13 (rabbit kidney). Virus stored in a freezing solution (refer to Chapter 3) at –80 °C was thawed at room temperature and 0,3 ml was inoculated into 25 cm^2 tissue cultures flasks. They were then incubated at 37 °C and checked daily until a 4+ cytopathic effect (CPE) was noted. Each flask was then snap-frozen at –80 °C and thawed at room temperature to release cell-associated virus. The viral suspension was re-cultured and snap-frozen and thawed once more to increase the viral numbers. One millilitre of this solution was again inoculated into a 150 cm^2 tissue culture flask and incubated at 37 °C until a 4+ CPE was noted. The flask was then snap-frozen at -80 °C and thawed at room temperature. It was centrifuged at 150 g for 2 minutes to sediment the cellular debris and 0,8 ml aliquots of supernatant were placed into 1 ml cryotubes and stored at –80 °C as reference stock virus until required.

Virus titration

To determine the concentration of stock virus in the solution a series of eight 10-fold dilutions of virus was prepared in sterile capped plastic test tubes by adding 4,5 ml cell culture medium to the all the tubes and 0,5 ml of the stock virus to the first tube designated 10^{-1}. Using a separate calibrated, glass pipette per dilution, thus avoiding virus carry-over, 0,5 ml of the virus solution was serially carried over to the next dilution until 10^{-8}, with the last 0,5 ml being discarded (refer to Figure I) Using a 96-well, flat-bottomed, microtitre plate with a lid (NUNC, Amersham), and a separate sterile pipette tip per dilution, triplicate 100 µl aliquots of each dilution were inoculated into separate wells. One hundred microlitres of cell culture medium and 80 µl of the prepared cell suspension was added to each well. Quintriplicate cell controls were set up by adding 80 µl of cells to 200 µl of cell culture medium. The plate was then incubated in a 5 % CO_2 incubator at 37 °C (refer to Figure 29).

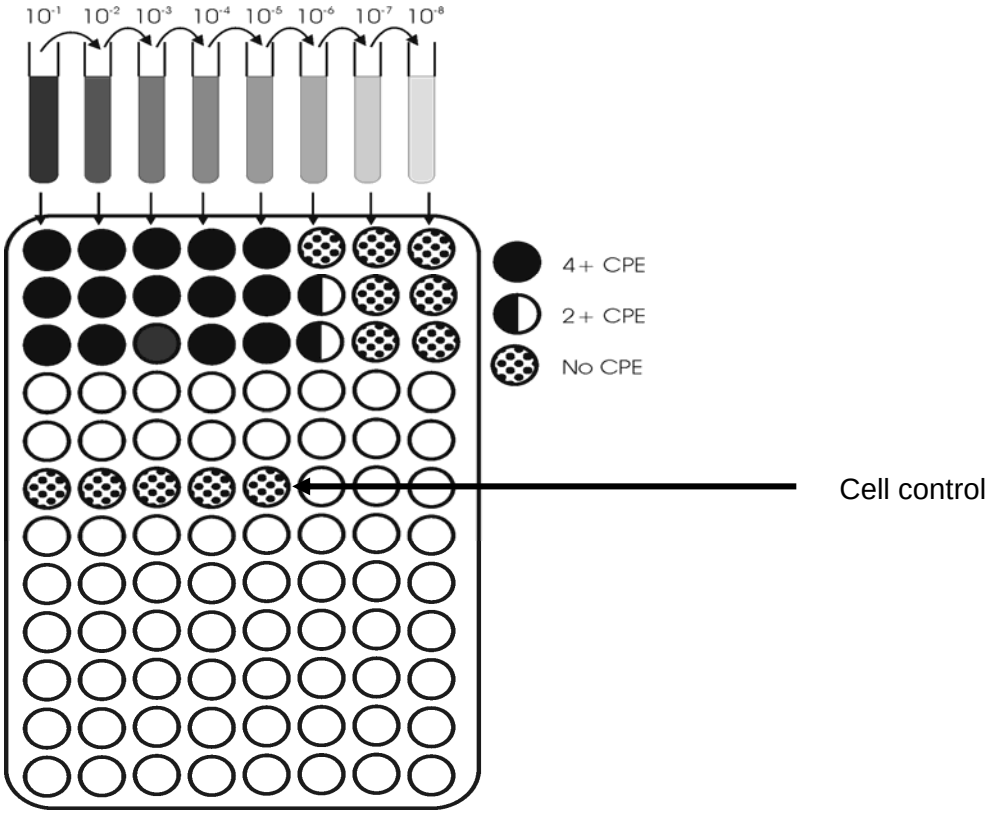

Figure 29: Diagrammatic representation of virus titration method.

The plates were read daily for about 7 days using an inverted compound microscope at a magnification of 200X until the cell control was still readable. One Tissue Culture Infective $Dose_{50}$ ($TCID_{50}$) of virus was taken as the virus dilution required to kill 50 % of the cells using the method of Reed & Muench (1939) (cited by Lennette 1969). One hundred $TCID_{50}$ of the virus was used to test for neutralizing antibodies in sera, which is equivalent to two back titrations of the $TCID_{50}$. The viral solution was then diluted in freezing solution (see Chapter 3) to a dilution of 100 $TCID_{50}$ in 80µl and 80 µl aliquots were placed in 1 ml cryotubes and frozen at –80 °C.

Test procedure

Using a multichannel pipette with sterile tips, serial 2-fold falling dilutions of serum were prepared in duplicate in 96-well, flat-bottomed, microtitre plates with lids. Using 100 µl viral culture medium and 5 % FCS as diluent, One hundred microlitres of inactivated 1:5 diluted serum was added to the first well and serial dilutions were made from 1:10 until 1:320. 100 µl was discarded from the last dilution. The test virus was added to each well at a concentration of 100 $TCID_{50}$/80 µl. Two hundred microlitres of viral culture medium containing 5 % FCS was added to 4 wells designated as the cell controls. A viral back titration was set up by making 4 serial ten-fold dilutions of 100 $TCID_{50}$ of the test virus in PBS+ in capped test tubes. One hundred microlitres of each virus dilution was added to 100 µl of virus culture medium containing 5 % FCS to each well. The plates were then sealed and incubated for 1 hour at 37

°C in a 5 % CO_2 in air incubator. After incubation 80 µl of the required cells were added to all wells.

The progress of the CPE was read daily using a 20X objective on an inverted compound microscope. All was recorded in a book on a 0 to 4 scale with 0 being no infection of cells, and 1, 2, 3 and 4 being approximately 25, 50, 75 and 100 % infection of cells. The end point was when the viral control showed mostly a reading of 4 at the dilution of 10^{-1} or less and 2 at the dilution of 10^{-2} and the cells in the cell control appeared to be healthy. The antibody titre was expressed as the reciprocal of the highest dilution of serum that gave 50% protection as calculated by the method of Reed & Muench (1938) (cited by Lennette, 1969).

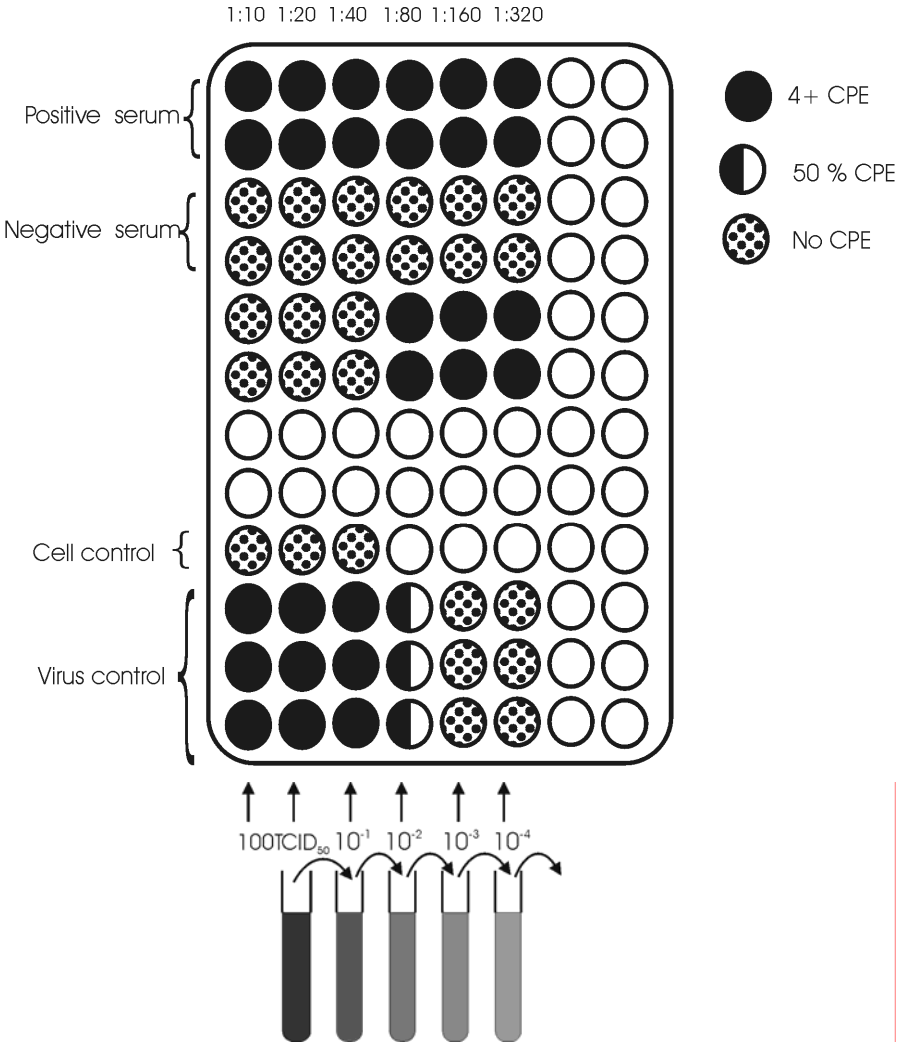

Figure 30: Diagrammatic representation of the serum neutralization test.

THE COMPLEMENT-FIXATION TEST

Preparation of erythrocyte cell suspension

Forty millilitres of blood was collected from an adult healthy sheep by jugular veni-puncture and mixed with 40 ml of Alsever's solution (see Appendix I). The blood was then stored for at least 3 to 5 days at 4 °C. The sheep erythrocytes (SRBC) were washed by placing 10 ml of the erythrocyte mixture in a graduated centrifuge tube and centrifuging at 1500 g for 10 minutes. The supernatant was removed and the cells re-suspended in VBS, mixed well and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded again and the process repeated three more times. A 2 % cell suspension was made by placing 2 ml of the packed cells into a measuring flask and adding 98 ml of VBS. For colorimetric standardization a 1:5 dilution of the SRBC suspension was made by lyzing 0,8 ml of the solution in 3,2 ml of deionised water. The optical density of the lyzed erythrocytes were measured at a wavelength of 550 nm in a spectrophotometer and the cell solution was adjusted to 0,6 optical density (OD).

Preparation of amboceptor

Commercially available anti-sheep erythrocyte antibodies (amboceptor) prepared in rabbits were used in these tests. A stock solution of 1:100 of amboceptor was made by rehydrating amboceptor (Virion) in I ml of sterile deionized water and mixing it with 99 ml of VBS. This stock solution was stored at 20 °C until required. On the day of use, it was diluted 1:25 in VBS to make a final dilution of 1:2500.

Preparation of the haemolytic system

It is prepared on the day it is required. An equal volume of a working dilution of amboceptor (1:2500) and 2 % SRBC suspension were used in the haemolytic system. This means that for each 96-well plate, 0,1 ml of stock amboceptor was added to 2,4 ml of VBS and then added to 2,5 ml of 2 per cent SRBC.

Preparation of complement

Heart blood was collected from guinea pigs that were fed a ration high in lucerne and allowed to clot. The serum was then decanted into 1,8 ml cryotubes and frozen at –80 °C. Guinea pigs fed rations high in lucerne tend to have more complement present in the serum than those not fed on this ration.

Concentration of complement determination

This was only done for each new batch of complement. As shown in Table 11, 0,1 ml of complement was added to 11 test tubes and the required dilution of VBS was added to each test tube.

Table 12: Dilution of complement to determine the concentration of complement to be used in the test.

Final dilution	1:10	1:20	1:25	1:30	1:35	1:40	1:45	1:50	1:55	1:60	1:65
Complement (ml)	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
VBS (ml)	0,9	1,9	2,4	2,9	3,4	3,9	4,4	4,9	5,4	5,9	6,4

Twenty-five microlitres of each dilution was transferred into a separate well of a 96-well, "U" bottomed, microtitre plate (Sterilin, Sterilab, SA). To all wells 25 µl of the working concentration of each antigen to be tested and 25 µl of VBS were added. The plate was sealed and incubated at 4 °C overnight. The next day 50 µl of the haemolytic system were added to each well and the plate was placed on a shaker at 37 °C for 20 minutes. The minimum haemolytic dose of complement (MHD) was considered to be the highest dilution of complement that completely haemolyzed the red blood cells. Twice this concentration (2 MHD) was used in the test and was known as the working complement concentration.

Complement back titration

For each batch of tests a back titration of complement was done. After the complement was diluted to 2 MHD, further dilutions were made in test tubes as follows: 0,3 ml VBS added to 0,9 ml of 2 MHD dilution to make 1.5 MHD, 0,6 ml VBS and 0,6 ml 2 MHD to make 1 MHD and 0,9 ml VBS added to 0,3 ml of 2 MHD to make a 0,5 dilution. Twenty-five microlitres of each dilution was added to the corresponding marked well that that contained 50 µl of VBS. After the plates had been incubated overnight at 4 °C, 50 µl of the haemolytic system was added as for the complement test procedure.

Optimal antigen concentration determination

Equid herpesvirus 1 and EHV-4 were prepared by growing them in primary lung cells according to the method described for the virus neutralization test. After a 4+ CPE was noted the culture flasks were snap-frozen at –80 °C and thawed at room temperature 3 times. The concentration of virus antigen per culture batch to be used in the test was determined as follows:

A so-called "Checkerbox" titration method was used. Serial dilutions of antigen were tested against serial dilutions of immune serum to determine the optimum dilution of antigen that gives fixation. A diagrammatic representation of the "checkerbox" titration method is represented in Table 12. After placing 0,25 µl of the diluent in each well of a 96 well, "U"-bottomed, microtitre plate, and adding 25 µl of serum to each of the wells in the first row, serial two-fold dilutions were made of the strongly positive immune inactivated sera from 1:5 to 1:640 using a multichannel pipette. The viral dilutions were made by making two series of serial dilutions of virus in VBS, one starting at 1:2 and ending at 1:16, and the other starting at 1:5 and ending at 1:20. Twenty-five microlitres of each virus dilution was then transferred to the wells as depicted in Table 12. Twenty-five microlitres of the working complement

concentration was added to each well. A back titration of complement was made as indicated in Table 12. Known negative serum and antigen controls as well as antigen from a previous titration were placed in the test as a control. The plate was carefully mixed, covered and incubated at 4 °C overnight. The next day 50 µl of the haemolytic system was added after the plates had been allowed to adjust to room temperature. The plate was then sealed and incubated at 37 °C in an incubator for 20 minutes. The results were read on a 0 to 4 scale, 4 being complete sedimentation (pellet formation) and 2 being 50 per cent sedimentation (a button about half the size as the 4 with some opacity to the fluid part of the well). The highest dilution of antigen with the highest dilution of serum where 50 % haemolysis occurred was considered to be one antigen unit. Two units of antigen were used in the test.

Table 13: The checkerbox method of titrating virus antigen against hyperimmune sera with a negative control serum control and complement control added. Within the box is an example of a possible result.

Antigen dilution	Immune serum dilutions								Negative serum	Complement control (units of complement)			
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:8	2.0†	1.5	1.0	0.5
1:2	4*	4	4	0	0	0	0		0	0	0	±	4
1:4	4	4	4	1	0	0	0		0	0	0	0	4
1:5	4	4	4	2	0	0	0		0	0	0	0	4
1:8	4	4	4	2	0	0	0		0	0	0	0	4
1:10	4	4	2	0	0	0	0		0	0	0	0	4
1:16	1	0	0	0	0	0	0		0	0	0	0	4
1:20	1	0	0	0	0	0	0		0	0	0	0	4
No antigen	0	0	0	0	0	0	0		0	0			
Non specific antigen	0	0	0	0	0	0	0		0	0	0	0	4
Previous lot of specific antigen	4	4	4	0	0	0	0		0	0	0	0	4

* Degree of fixation on a 0 to 4 scale.
† Working dilution of complement.

Preparation of test serum

The sera to be tested as well as the known positive and negative sera controls were diluted 1:5, i.e. 0,5 ml serum was added to 2 ml PBS+ and incubated for 30 minutes in a waterbath at 56 °C to inactivate anti-complementary factors in the serum.

Test procedure

A known high positive serum and negative serum to the viruses to be tested were incorporated as positive and negative controls. Two rows of a 96-well, “U”-bottomed, microtitre plate were allocated per serum, one to which antigen was added and the other to which no antigen was added as a control to check for anti-complementary activity in the sera.

Fifty microlitres of the inactivated sera were added to the first column and thereafter serial two-fold dilutions were made in each row from column two using 25 µl of serum from the first well and 25 µl VBS using a multichannel pipette. Twenty-five microlitres containing 2 antigen units of viral antigen was added to each well of the first row and 25 µl of VBS to the second row of each test serum. Twenty-five microlitres of 2 MHD of guinea pig complement was then added to all wells. For each test a duplicate back titration of complement was done using 2 (working dilution), 1,5, 1, and 0,5 units of complement, thereafter adding 50 µl VBS and 25 µl virus antigen to all the wells. The plates were then sealed and incubated overnight at 4 °C. The next day, the microtitre plates were allowed to warm to room temperature. Then 50 µl of the haemolytic system was added to all wells and the plates were placed on a shaker. They were incubated at 37 °C for 20 minutes to ensure contact of virus-antibody complex with the erythrocytes. The test was considered to be complete when the back titration results were as follows: 0,5 unit wells, no haemolysis (button formation); one unit wells, slight haemolysis; 1,5 and 2 unit wells, total haemolysis. The plates were then removed from the incubator and centrifuged for 10 minutes at 150 g. The antibody titre was expressed as the reciprocal of the highest dilution that caused a 50 % haemolysis of the erythrocytes.

APPENDIX III: BACTERIA CULTURED FROM THE NASOPHARYNX OF FOALS

Foal	21/09/1993	26/10/1993	23/11/93	14/12/93	20/01/94	24/02/94	29/03/94	02/04/94
F1	Not born	*Staphylococcus aureus* *Staphylococcus* spp. x 2	No growth	*S. aureus* *Staphylococcus* sp. *Corynebacterium* spp. x 2 *Bacillus* spp. (x 2)	*Actinobacillus equuli* *Staphylococcus* spp. *Enterococcus* sp. *Corynebacterium* sp. *Escherihica coli* (rough) *Pantoea agglomerans*	No growth	*S. aureus* *Staphylococcus* sp. *Proteus mirabilis* *Flavobacterium* sp.	*Staphylococcus* spp. x 2 *Moraxella* spp.
F2	Not born	*Staphylococcus* spp. *Moraxella* sp. *Flavobacterium* sp.	*A. equuli* Viridans strep.	A. equuli *Staphylococcus* spp. *Enterococcus faecalis* *Acinetobacter anitratus*	*Staphylococcus* spp. *Nocardia* sp. *Flavobacterium* sp. *Burkholderia cepacia*	*Staphylococcus* sp. *Streptomyces* sp. *Corynebacterium* sp.	Not done	*Streptococcus zooepidemicus* *Staphylococcus* spp. x 2 *Corynebacterium* sp. *Aeromonas hydrophila* *Moraxella* sp.
F3	Not born	Not taken	*S. zooepidemicus* *Enterococcus* spp. *Bacillus* sp. *P. agglomerans*	Viridans strep. *Mannheimia haemolytica* Bacilllus cereus	*Staphylococcus* sp. *B. cereus* *Corynebacterium* sp.	No growth	*Staphylococcus* spp. x 2 Viridans strep. *Bacillus* sp. *B. cepacia*	Not done
F5	Not born	*Staphylococcus* spp. x 2 *Streptococcus agalactiae* *Oeskovia* sp. *Kurthia* sp.	No growth	*A. equuli* Viridans strep. *A. anitratus* *Pasteurella* sp. *Bacillus* sp.	*Staphylococcus* sp. *Enterococcus* sp. *Bacillus* spp. x2 *Kurthia* sp. *Streptobacillus* sp.	*S. aureus* *Staphylococcus* spp. x 2	Not done	*S. zooepidemicus* *S. aureus* *Staphylococcus* spp. x 2 *Enterococcus* sp. Viridans strep.
F6	Not born	*Staphylococcus* spp. x 2 *Proteus vulgaris* *Bacillus* sp.	*A. equuli* *Flavobacterium* sp.	*A. equuli* Viridans strep. *A. anitratus* *Pasteurella* sp. *Bacillus* sp.	*A. acetinomyceto** *Actinobacillus lignieresii*	No growth	*S. aureus* *Staphylococcus* spp. x 4 *Bacillus* sp.	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep.
F8	Not born	*Staphylococcus* sp. *Corynebacterium* sp. *Agrabacterium* sp. *Bacillus* sp.	*A. equuli* *S. zooepid*emicus *Enterococcus* spp. *Staphylococcus* sp. *Bacillus* sp.	*A. equuli* *Staphylococcus* sp. *Flavobacterium* sp. *Bacillus* sp.	*Staphylococcus* spp. x2 *P. mirabilis* *Flavobacterium* sp. *Bacillus* sp.	*S. zooepidemicus*	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep.	Not done
F9	*Micrococcus* spp. x 2 *Corynebacterium* sp. *Aerococcus* sp. *Streptomyces* sp.	*Staphylococcus* spp. x 2 Viridans strep. *A. anitratus*	A. equuli Viridans strep. *Staphylococcus* sp. *Corynebacterium* sp.	*Staphylococcus* spp. x 4 Viridans strep. *A. anitratus*	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep. *Corynebacterium* sp. *B. cereus* *Oeskovia* sp. *Nocardia* sp. *A. lignieresii* *P. agglomerans*	*Staphylococcus* sp. *Corynebacterium* sp. *Serratia rubidea*	*S. zooepidemicus* *Corynebacterium* sp. *Rhodococcus* sp. *Streptomyces* sp. *Moraxella* sp.	*S. aureus* *Staphylococcus* spp. x 3 *Corynebacterium* sp. *Moraxella* sp.

* *A. acetinomyce = Actinobacilllus acetinomycetocomitans*
\# virudans strep. = viridans streptococcus

Foal	21/09/1993	26/10/1993	23/11/93	14/12/93	20/01/94	24/02/94	29/03/94	02/04/94
F11	Not born	*A. equuli* *Aerococcus* sp. *Streptomyces* sp *Pseudomonas maltophilia* *Flavobacterium* sp.	*S. zooepidemicus* *Bacillus* sp. *Moraxella* sp. *Streptobacillus* sp.	*A. equuli* *Staphylococcus* sp. *Flavobacterium* sp. *Moraxella* sp.	*S. zooepidemicus* *S. aureus* *Staphylococcus* spp. x 2 Viridans strep. *Enterococcus* sp. *Bacillus* sp. *E. coli* (rough) *Proteus* sp. *Streptobacillus* sp.	No growth	*S. aureus* *Staphylococcus* sp. *Corynebacterium* sp. *Streptomyces* sp.	*Staphylococcus* sp. *Corynebacterium* sp. *Moraxella* sp. *Bacillus* spp. X2
F13	Not born	*Staphylococcus* sp. Viridans strep. *Micrococcus* sp. *B. cepacia*	*S. zooepidemicus* Viridans strep. *Enterococcus* sp. *Klebsiella pneumoniae*	*A. equuli* *E. coli* (rough) *Moraxella* sp. *Bacillus* spp. x2	*S. zooepidemicus* *A. equuli* *Enterococcus* sp. *Corynebacterium* sp. *A. c. wolffii*	No growth	*A. equuli* *S. zooepidemicus* *Staphylococcus* sp. Viridans strep. *Moraxella* sp.	*S. aureus* *Staphylococcus* sp. *Nocardia* sp. *Streptobacillus* sp.
F15	Not born	*Staphylococcus* spp. x 2 P. vulgaris	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep.	*S. zooepidemicus* *Staphylococcus* spp. x2 *Bacillus* sp.	*A. equuli* *S. zooepidemicus* *Flavobacterium* sp. *Bacillus* sp. *Moraxella* sp.	*Staphylococcus* sp. Viridans strep. *Nocardia* sp. *B. cepacia* *Moraxella* sp.	*A. equuli* *Staphylococcus* sp. Viridans strep. *B. cepacia*	*S. aureus* *Staphylococcus* spp. x 3 *Corynebacterium* sp. *Enterococcus* sp. *Flavobacterium* sp.
F16	Not born	*Pneumococcus* sp. *Bacillus* sp. *Flavobacterium* sp. *Moraxella* sp.	*A. equuli* *Pasteurella* sp. *Streptomyces* sp.	*Staphylococcus* sp. *Enterococcus* sp. *E. coli* (rough) S. equinus	*A. equuli* *S. zooepidemicus* *Staphylococcus* sp. *Aerococcus* sp. *Flavobacterium* sp. *Bacillus* sp.	No growth	*A. equuli* *Staphylococcus* sp. *Corynebacterium* sp.	*A. equuli* *S. zooepidemicus* *S. aureus* *Staphylococcus* spp. x 3 *Bacillus* sp. *Flavobacterium* sp.
F17	*Staphylococcus* sp. *Agrabacterium radiatium* *Corynebacterium* sp. *Bacillus* sp.	Viridans strep. *Bacillus* sp.	*S. zooepidemicus* *A. lignieresii* *Staphylococcus* sp. *Enterococcus* sp. *Streptobacillus* sp.	*A. equuli* *S. zooepidemicus*	*A. equuli* *S. zooepidemicus* *Staphylococcus* spp. x3 *Bacillus* sp. *Ps. cepacia*	Not done	Viridans strep. *B. cepacia* *Moraxella* sp.	*Staphylococcus* sp. *Moraxella* sp. *Neisseria* sp.
F18	*Staphylococcus* spp. x2 *Bacillus* sp. *P. rettgeri*	*Staphylococcus* sp. Viridans strep.	*S. zooepidemicus* *Bacillus* spp. x2 *Streptobacillus* sp.	*S. zooepidemicus* *A. wolffii* *Bacillus* sp. *Flavobacterium* sp.	*Staphylococcus* sp. Viridans strep. *Nocardia* sp. *Streptobacillus* sp.	*S. intermedius* *B. cepacia* *Flavobacterium* sp.	*Staphylococcus* spp. x2 *Flavobacterium* sp.	*S. aureus* *Corynebacterium* sp. *A. hydrophila* *B. cepacia* *Moraxella* sp. *Flavobacterium* sp.
F19	Not born	*Staphylococcus* spp. x2 Viridans strep. *Corynebacterium* spp. x2 *Rhodococcus* sp.	*Staphylococcus* sp. *Bacillus* sp. P. rettgeri *Streptobacillus* sp. *Streptomyces* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Bacillus* sp. *Pasteurella* sp.	A. equuli *Enterococcus* sp. *A. c. wolffii* *E. agglomerans* *Bacillus* sp. *Flavobacterium* sp. *Streptobacillus* sp.	*S. zooepidemicus* *Staphylococcus* spp. x2 *Moraxella* sp.	*Staphylococcus* spp. x2 Viridans strep. *Moraxella* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Moraxella* sp.

Foal	21/09/1993	26/10/1993	23/11/93	14/12/93	20/01/94	24/02/94	29/03/94	02/04/94
F20	Not born	*Staphylococcus* sp. *Enterococcus* sp. *Bacillus* sp.	*Staphylococcus* sp. S. equinus *Streptobacillus* sp.	*S. aureus* *Staphylococcus* sp. *Pasteurella* sp.	*A. wolffii* *P. mirabilis* *Bacillus* sp. *Streptobacillus* sp.	No growth	*S. zooepidemicus*	*S. zooepidemicus* *S. aureus*
F22	Not born	Viridans strep. *E. coli* (rough)	Viridans strep. *Flavobacterium* sp. *Streptobacillus* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Enterococcus* sp. *Bacillus* sp. *A. wolffii*	*Staphylococcus* spp. x2 Viridans strep. *Aerococcus* sp. *Moraxella* sp.	Not done	*S. zooepidemicus* *Staphylococcus* spp. x2 *Neisseria* sp.	*Staphylococcus* sp. *Moraxella* sp. *Flavobacterium* sp.
F23	*Micrococcus* spp. x2 *Aerococcus* sp. *Corynebacterium* sp.	*A. equuli* *Oeskovia* sp. *Flavobacterium* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Corynebacterium* sp. *Streptobacillus* sp.	*Staphylococcus* sp. *Flavobacterium* sp. *Streptobacillus* sp. *Streptomyces* sp.	*A. c. wolffii* *Staphylococcus* spp. x2	No growth	*S. zooepidemicus* Viridans strep. x2	*S. aureus* *Staphylococcus* spp. x2 *Bacillus* sp. *Streptomyces* sp.
F24	Not born	*Staphylococcus* sp. *Enterococcus* sp. *E. coli* (rough)	*A. equuli* Viridans strep.	*Staphylococcus* sp. *Streptobacillus* sp.	*Staphylococcus* spp. x2 *Aerococcus* sp. *Bacillus* sp. *Corynebacterium* sp. *Oeskovia* sp.	Not done	*Staphylococcus* sp. Viridans strep. *Flavobacterium* sp. *Moraxella* sp.	Not done
F25	Not born	Viridans strep. *E. coli* (rough)	*S. zooepidemicus* Viridans streptococci x2 *P. mirabilis* *Streptobacillus* sp.	A. equuli *S. zooepidemicus* *A. wolffii* *Bacillus* sp.	*Corynebacterium* sp. *Listeria* sp. *Streptobacillus* sp.	*P. mirabilis*	*A. lignieresii* *Moraxella* sp.	*S. aureus* Staphylococcus *sp.* *P. vulgaris* *Moraxella* sp. B. cepacia
F26	*B. cepacia* *Moraxella* sp. *P. mirabilis*	*Staphylococcus* spp. x3 *Enterococcus* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Bacillus* sp. *Streptobacillus* sp.	*Staphylococcus* sp. *Corynebacterium* sp. *A. wolffii* *Pasteurella* sp. *Flavobacterium* sp.	*S. zooepidemicus* *A.lignieresii* *Staphylococcus* spp. x2 *Corynebacterium* sp. *Bacillus* sp. *Moraxella* sp.	*A. equuli* *Staphylococcus* sp. *Bacillus* sp. *Flavobacterium* sp.	*A. equuli* *S. aureus* *Corynebacterium* sp. *Flavobacterium* sp. *B. cepacia*	*Staphylococcus* sp. *P. vulgaris* *A. hydrophila*
F29	Not born	*A. equuli*	*S. aureus* *Staphylococcus* sp. *Moraxella* sp	*A. lignieresii* *Corynebacterium* sp. *Bacillus* sp. *Pasteurella* sp.	*Staphylococcus* spp. x2 Viridans strep. *Flavobacterium* sp	*Staphylococcus* spp. x2 Viridans strep. *Streptomyces* sp.	*A. equuli* *Staphylococcus* sp. *Corynebacterium* sp.	*Staphylococcus* sp. *Moraxella* sp.
F30	Not born	Not born	*S. zooepidemicus* *Enterococcus* sp. *Moraxella* sp. *Passteurella* sp.	*A. equuli* *Pasteurella* sp.	Viridans strep. *Bacillus* sp. *A. wolffii* *Pasteurella* sp. *Flavobacterium* sp. *Streptobacillus* sp.	*Staphylococcus* sp. Viridans strep.	*A. equuli* *Staphylococcus* sp. *Bacillus* sp. P. rettgeri *Moraxella* sp.	*S. zooepidemicus* *S. aureus* *Staphylococcus* sp. *Corynebacterium* sp. *Nocardia* sp.
F31	Not born	*S. zooepidemicus* *Staphylococcus* spp. x2 *P. mirabilis*	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep. *Streptobacillus* sp.	*A. equuli* *Staphylococcus* sp. *Bacillus* sp. *Moraxella* sp. *A. wolffii*	*S. zooepidemicus* *Aerococcus* sp. *Moraxella* sp.	*Staphylococcus* sp. *Bacillus* sp. *Corynebacterium* sp.	*A. equuli* *S. zooepidemicus* *Staphylococcus* sp.	Not done

Foal	21/09/1993	26/10/1993	23/11/93	14/12/93	20/01/94	24/02/94	29/03/94	02/04/94
F32	Not born	*Staphylococcus* spp. x2 *Bacillus* sp. *A. anitratus* *S. rubidaea*	*A. equuli* *Corynebacterium* sp.	*A. equuli* *Staphylococcus* sp.	*A. equuli* *Staphylococcus* sp. *P. mirabilis*	No growth	Viridans strep. *Corynebacterium* sp. *Moraxella* sp. *Flavobacterium* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Moraxella* sp.
F33	Not born	Not born	*S. zooepidemicus* *Staphylococcus* sp. *Streptobacillus* sp.	*Nocardia* sp. *Pasteurella* sp. *Moraxella* sp. *Flavobacterium* sp.	*A. equuli* *Flavobacterium* sp.	No growth	*S. aureus* *Staphylococcus* sp. *Bacillus* sp. *Moraxella* sp.	*Staphylococcus* sp. *Moraxella* sp. *Flavobacterium* sp.
F34	Not born	*A. equuli* Viridans streptococci	KwaZulu-Natal	KwaZulu-Natal	*S. aureus* *P. mirabilis* *Streptobacillus* sp.	No growth	*S. zooepidemicus* *S. aureus* *Staphylococcus* sp. *Pasteurella* sp.	*Staphylococcus* spp. x2 Viridans strep. *Micrococcus* sp. *Nocardia* sp. *Bacillus* sp. *Moraxella* sp. *Pasteurella* sp.
F35	Not born	*A. equuli* *Bacillus sp.*	Western Cape	Western Cape	*S. zooepidemicus* *Staphylococcus* spp. x2 *Moraxella* sp. *Flavobacterium* sp.	Not done	Not done	*Staphylococcus* spp. x3 Viridans strep. *Bacillus* sp. *Moraxella* sp. *A. acetinomyce**
F37	Not born	*Staphylococcus* spp. x2 *Corynebacterium* sp. *Bacillus* sp.	*Staphylococcus* spp. x2 *Corynebacterium* sp. *Bacillus* sp. *Streptobacillus* sp.	*A. equuli* *Staphylococcus* sp. *Pasteurella* sp.	*Staphylococcus* sp. *Corynebacterium* sp. *Nocardia* sp. *Bacillus* sp. *E. coli* (rough) *A. wolffii* *B. cepacia*	*A. equuli* *S. aureus* *Corynebacterium* spp. x2 *Enterobacter* sp. *Flavobacterium* sp.	*A. equuli* *S. aureus* *Moraxella* sp. *A. acetinomyce**	*S. aureus* *Staphylococcus* sp. *Corynebacterium* sp. *Pseudomonas* sp. *Flavobacterium* sp.
F38	Not born	Not born	*Staphylococcus* sp. *Streptobacillus* sp.	*A. equuli* *Staphylococcus* spp. x2 *Micrococcus* sp. *Corynebacterium* sp. *Moraxella* sp. *Streptomyces* sp.	*A. equuli* *B. cepacia*	No growth	*A. equuli* *S. zooepidemicus* *Staphylococcus* sp. Viridans strep.	Not done
F39	Not born	*Staphylococcus* sp.	*Corynebacterium* sp. *Streptobacillus* sp.	*A. equuli* *Corynebacterium* sp.	*Flavobacterium* spp. x2	*S. aureus* *Corynebacterium* sp. *Bacillus* sp. *Moraxella* sp.	*A. equuli* *S. zooepidemicus* *Staphylococcus* sp. *Moraxella* sp.	*S. aureus* *Staphylococcus* spp. x2 Viridans strep. *Corynebacterium* sp.

Foal	4/05/94	2/06/94	27/06/94	2/08/94
F1	*A. equuli* *Staphylococcus* sp. *Moraxella* sp.	Viridans strep. *Enterococcus* sp. *Corynebacterium* sp. *Bacillus* spp. x2 *Streptomyces* sp. *B. cepacia* *Moraxella* sp. *Pasteurella* sp. *Flavobacterium* sp.	*S. zooepidemicus* *S. aureus* *Staphylococcus* spp. x 2 Viridans strep.	*Staphylococcus* spp. x 4 *Corynebacterium* sp.
F2	*S. zooepidemicus* *S. aureus* *Staphylococcus* spp. x2 *Corynebacterium* sp. *Moraxella* sp.	*S. zooepidemicus* *Staphylococcus* sp. Viridans strep. *Bacillus* sp. *Moraxella* sp. *Pseudomonas* sp. *Flavobacterium* sp.	*Staphylococcus* spp. x 2 *Corynebacterium* sp. *E. coli* (rough) *Moraxella* sp.	*M. haemolytica* *Virudans strep.* *E. faecalis* *P. vulgaris* *P. agglomerans* *Moraxella* sp.
F3	*S. zooepidemicus* *S. aureus* *Staphylococcus* sp.	*S. zooepidemicus* Viridans strep. *Bacillus* sp. *Streptomyces* sp. *A. wolffii* *B. cepacia* *Pasteurella* sp. *Flavobacterium* sp. *Streptobacillus* sp.	*Bacillus* sp. *Moraxella* sp.	*Staphylococcus* spp. x 3 *Micrococcus* species Viridans strep. *Corynebacterium* sp.
F5	*S. zooepidemicus* *S. aureus* *Staphylococcus* sp.	*S. zooepidemicus* *Flavobacterium* sp.	*S. intermedius* *Staphylococcus* spp. Viridans strep. *B. cereus* *Streptomyces* sp. *A. anitratus*.	S. zooepidemicus Viridans strep. *A. anitratus*. *P. agglomerans*
F6	*S. zooepidemicus* *Staphylococcus* sp.	S. zooepidemicus *Bacillus* sp. *Pasteurella* sp. *Moraxella* spp. x2	*S. zooepidemicus* *S. aureus* *Bacillus* sp. *Corynebacterium* sp. *E. coli* (rough)	*S. zooepidemicus* *Corynebacterium* sp. *P. vulgaris*
F8	Viridans strep. *Staphylococcus* sp. *Moraxella* sp.	*S. zooepidemicus* *Pseudomonas* sp.	*Staphylococcus* sp. Virudans strep. *E. coli* (rough)	*Staphylococcus* spp. x 2 *B. cepacia* *Moraxella* sp.
F9	Viridans strep. *Corynebacterium* sp. *Moraxella* sp.	*Staphylococcus* spp. x2 Viridans strep. *Corynebacterium* spp. x2 *P. mirabilis* *Pasteurella* sp. *Moraxella* sp. *Flavobacterium* sp. *Streptomyces* sp.	*S. zooepidemicus* *S. intermedius* *Staphylococcus* sp. *S. equinus*	*Staphylococcus* spp. x 2 Viridans strep. *Pasteurella* sp.

Foal	4/05/94	2/06/94	27/06/94	2/08/94
F11	*S. aureus* *Bacillus* sp. *Corynebacterium* sp. *Moraxella* sp.	*S. zooepidemicus* *Staphylococcus* spp. x2 *Corynebacterium* spp. x2 *Bacillus* spp. x3 *Flavobacterium* spp. x3 *Simonsiella* sp.	*S. zooepidemicus* *Bacillus* sp. *Staphylococcus* sp. X 2 *Flavobacterium* sp. *A. wolfii* *A. hydrophila* *Moraxella* sp.	*S. aureus* *Staphylococcus* sp. X 3 *Bacillus* sp. *Moraxella* sp.
F13	*A. equuli* *S. aureus* *Staphylococcus* sp. *Streptomyces* sp.	*S. aureus* *Staphylococcus* spp. *x2* *Pasteurella* sp. *Moraxella* sp.	S. zooepidemicus *Staphylococcus* sp. X 2 *Bacillus* sp. *Pseudomonas* sp. *P. multocida*	*Staphylococcus* sp. X 5 *Flavobacterium* sp. *A. hydrophila*
F15	*S. aureus* *Staphylococcus* sp. *Corynebacterium* sp. *Streptococcus* sp. *Flavobacterium* sp. *Pasteurella* sp.	*A. equuli* *S. aureus* *Corynebacterium* sp. x2 *Pasteurella* sp. *Proteus* sp.	*S. aureus* Virudans strep. *A. anitratus*	*S. zooepidemicus Staphylococcus* sp. Viridans strep. *Bacillus* sp.
F16	*S. zooepidemicus* *S. aureus* *M. haemolytica* *Staphylococcus* sp. *Corynebacterium* sp. *Streptococcus* sp. *Moraxella* sp.	*A. equuli* *S. aureus* *Staphylococcus* spp. x2 *Pasteurella* sp.	*Staphylococcus* spp. x2 Virudans strep. *Corynebacterium* sp. *Pasteurella* sp. *Pseudomonas* sp.	*S. zooepidemicus* *Staphylococcus* spp. X3 *Kurthia* sp. *Neisseria* sp.
F17	*A. equuli* *S. aureus* *Bacillus* sp. *Neisseria* sp.	*S. intermedius* *Streptomyces* sp. *Corynebacterium* sp. *B. cepacia* *A. wolffii* *Flavobacterium* sp.	*S. aureus* *P. agglomerans*	*Staphylococcus* sp. *B. cepacia* *Pasteurella* sp.
F18	*S. aureus* *S. zooepidemicus* *Corynebacterium* sp. Viridans strep. *Aeromonas hydrophila* *Streptobacillus* sp. *Moraxella* sp.	*S. zooepidemicus* *B. cereus* *Moraxella* sp. *Pasteurella* sp. *Enterobacter cloacae* *Flavobacterium* sp.	*B. cereus* *Streptobacillus* sp. *Flavobacterium* sp.	*Staphylococcus* spp. X3 *Pasteurella* sp. *Moraxella* sp. *E. coli* (rough)
F19	*Staphylococcus* spp. X 2 Viridans strep. *Moraxella* sp.	*A. equuli* *S. aureus* *Staphylococcus* sp. Viridans streptococc1 *Corynebacterium* sp. x2 *B. cepacia*	*S. zooepidemicus* Staphylococcus *sp.* Bacillus *sp.* *Flavobacterium* sp. *Moraxella* sp.	*Staphylococcus* sp. *Micrococcus* sp. *Bacillus* sp. *P. multocida* *A. hydrophila* *M. haemolytica* *Moraxella* sp.

Foal	4/05/94	2/06/94	27/06/94	2/08/94
F20	*S. zooepidemicus* *S. aureus* *Staphylococcus* spp. X 2	*A. equuli* *S. zooepidemicus* *E. coli* (rough) *Moraxella* sp.	*S. zooepidemicus* *Moraxella* sp.	*S. zooepidemicus* *Staphylococcus* spp. x 3 *Streptobacillus* species *Flavobacterium* sp. *Moraxella* sp.
F22	*Staphylococcus* sp. *Moraxella* sp. *Flavobacterium* sp.	*S. zooepidemicus* *Staphylococcus* sp. Viridans streptococci x2 *E. coli* (rough) *B. cepacia* *Simonsiella* sp.	*Staphylococcus* sp. x2 Viridans streptococci x 2 *B. cepacia*	*P. caballi* *S. aureus* *Staphylococcus* sp. x 5 *Micrococcus* sp. *E. faecalis*
F23	*Staphylococcus* spp. X2 *Bacillus* sp. *Streptobacillus* sp.	*S. zooepidemicus* *Corynebacterium* sp. *E. coli* (rough) *E. cloacae* *Streptobacillus* sp.	*Staphylococcus* sp. *Corynebacterium* sp. *Streptobacillus* sp.	*Micrococcus* sp. *Staphylococcus* sp. *Moraxella* sp.
F24	*S. zooepidemicus* *Moraxella* sp.	*S. zooepidemicus* *E. coli* (rough) *Moraxella* sp.	*S. zooepidemicus* *Moraxella* sp. *Flavobacterium* sp.	*S. zooepidemicus* *Staphylococcus* spp. x2 *Bacillus* sp. *Moraxella* sp. *Flavobacterium* sp.
F25	*A. equuli* *S. aureus* *Staphylococcus* sp. *P. mirabilis* *Pseudomonas* sp.	*Staphylococcus* sp. Viridans strep. *E. coli* *P. rettgeri* *Moraxella* sp.	*S. zooepidemicus* *Moraxella* sp. *Streptobacillus sp.*	*Staphylococcus* spp. x 3 *K. pneumoniae*
F26	*Staphylococcus* sp. *B. cereus* *A. hydrophila* *P. mirabilis*	*S. zooepidemicus* Viridans strep. *Pasteurella* sp. *P. rettgeri* *Flavobacterium* sp. *Moraxella* sp.	*S. zooepidemicus* *Moraxella* sp.	*S. zooepidemicus* *Staphylococcus* sp. *Moraxella* sp.
F29	*Staphylococcus* sp. *Moraxella* sp.	*S. aureus* *Corynebacterium* spp. x2 *Bacillus* sp. *Actinobacillus* sp. *Moraxella* sp. *A. wolffii* *Streptomyces* sp.	*S. zooepidemicus* *Staphylococcus* sp. x 2 *S. equinus* *E. coli* (rough)	*Staphylococcus* sp. x 2 *E. coli* (rough) *P. agglomerans*
F30	*S. zooepidemicus* *S. aureus* *Staphylococcus* sp. *Corynebacterium* sp. *Streptomyces* sp.	Viridans strep. *Corynebacterium* sp *Pasteurella* sp. *Flavobacterium* sp. *Streptobacillus* sp.	*S. intermedius* *Staphylococcus* spp. x3 *Micrococcus* sp. A. hydrophila	*Staphylococcus* sp. x2

Foal	4/05/94	2/06/94	27/06/94	2/08/94
F31	*S. zooepidemicus* Viridans strept. *B. cereus* *A. wolffii*	Not done	*E. coli* (rough) *Moraxella* sp.	Not done
F32	*S. zooepidemicus*	*S. zooepidemicus* Viridans strept. *P. rettgeri* *Moraxella* sp. *Flavobacterium* sp.	*Staphylococcus* sp. *Corynebacterium* sp *Flavobacterium* sp. *Moraxella* sp.	*S. zooepidemicus* *Enterococcus* species Viridans strept.
F33	*Staphylococcus* sp. *Nocardia* sp. *Flavobacterium* sp. *Moraxella* sp.	*Staphylococcus* sp. *B. cereus* *Corynebacterium* sp *Flavobacterium* sp. *Moraxella* sp. *Streptomyces* sp.	*Staphylococcus* sp. *Nocardia* sp.	*S. aureus* *S. zooepidemicus* *Enterococcus* species Viridans strept.
F34	*S. aureus* *Staphylococcus* sp. X3 *Nocardia* sp. *B. cereus* *Flavobacterium* species *Streptomyces* sp.	*S. zooepidemicus* *Staphylococcus* sp. Viridans strept. *Enterococcus* sp. *Corynebacterium* sp. *Streptobacillus* sp. *Streptomyces* sp. *Moraxella* sp.	*S. aureus* *Staphylococcus* sp. x 2 *Streptomyces* sp.	*S. aureus* *S. zooepidemicus* *S. intermedius* *Staphylococcus* sp. *Enterococcus* sp.
F35	*S. aureus* *Staphylococcus* spp. X2 B. cereus *Moraxella* sp.	*S. aureus* *Bacillus* sp. *Staphylococcus* sp. x3 *Streptobacillus* sp.	*Staphylococcus* sp. x3 *Moraxella* sp.	*S. aureus* *S. intermedius* *Bacillus* sp.
F37	*S. zooepidemicus* *S. aureus* *Corynebacterium* sp. X2 *B. cereus* *Pseudomonas* sp. *Flavobacterium* sp.	*S. zooepidemicus* Streptococcus *sp.* *Enterococcus sp.* *B. cereus* *Streptomyces* sp.	*S. zooepidemicus* *Enterococcus sp.* *Streptobacillus* sp. Pseudomonas *sp.*	*S. aureus* *S. zooepidemicus* *B. cereus*
F38	*Not done*		*Staphylococcus* sp. x 5 *Bacillus* sp.	*S. aureus* *Micrococcus* sp. *E. faecalis*
F39	*S. aureus* *S. intermedius* *Corynebacterium* sp.	*A. equuli* *S. zooepidemicus* *Streptococcus* sp. *Acinetobacter* sp. Streptobacillus *sp.*	*S. zooepidemicus Nocardia* sp. *Moraxella* sp.	*Corynebacterium* sp. *Micrococcus* sp. *E. faecalis* *Staphylococcus* sp. *A. wolfii* *Streptobacillus* sp.

APPENDIX IV: FUNGI ISOLATED FROM THE NASOPHARYNX OF FOALS

Foal	21/09 93	26/10/1993	23/11/93	24/12/93	20/01/94	24/02/94	29/03/94
F1	Not born	*Aspergillus glaucus* *A. niger* *Cladosporium* sp. *Mucor* sp.	*Cladophialophora* sp.	*Cladosporium* sp. *Fusarium* sp.	Cladophialophora sp. Trichophyton meganinii *Candida* sp.	*Cladophialophora* sp.	*Alternaria* sp. *A. flavus* *A. glaucus* *Cladophialophora* sp. Exophiala *sp.*
F2	Not born	No growth	*Cladophialophora* sp. *Geotrichium* sp. *Epicoccum* sp.	*Mucor* sp. T. ajello	Alternaria *sp.* Chrysosporium *sp.* Cladophialophora sp. *Monosporium* sp. T. schoenlenii	*A. flavus* *Cladophialophora* sp.	Not done
F3	Not born	Not born	Not born	Alternaria *sp.* A. glacus *A. fumigatus*	Not done	No growth	Alternaria *spp. x2*
F5	Not born	A. fumigatus *A. versicolor*	*Candida* sp.	*Cladophialophora* sp. *Rhodotorula* sp.	No growth	*Cladophialophora* spp .x2 T. rubrum *Rhodotorula* sp.	Not done
F6	Not born	No growth	No growth	Curvularia sp. *Botrytis* sp. *Fonseceae* sp.	*Alternaria* sp. *Cladosporium* sp. *Mucor* sp.	*Cladosporium* sp. *Fusarium* sp.	*Alternaria* sp. *A. terreus* *Cladosporium* sp. *Fusarium* sp.
F8	Not born	No growth	No growth	*Cladosporium* sp. *Alternaria* sp. A. flavus	*Candida* sp. *Cladosporium* sp. T. ajello	*A. flavus* *Cladosporium* sp.	*A. fumigatus* *A. glaucus* *A. nidulans* *Cladophialophora* sp. *Curvularia* sp.
F9	A. flavus	A. flavus	No growth	*Cladophialophora* sp.	*Mucor* sp.	*Alternaria* sp. *A. flavus* *A. terreus* *Cladophialophora* sp.	*Alternaria* sp. *Cladophialophora* sp.
F11	No growth	*A. niger* *Scopuloriopsis* sp. *T. ajello*	Aurobasidium pullulans Alternaria *sp.*	No growth	*Cladophialophora* sp. *Fusarium* sp. *Mucor* sp. T. meganinii *T. tonsurans*	No growth	*Alternaria* sp. *A. terreus*

Foal	21/09 93	26/10/1993	23/11/93	24/12/93	20/01/94	24/02/94	29/03/94
F13	Not born	*No growth*	Cladophialophora sp. *Alternaria* sp. *Epicoccum* sp. *Helminthosporium* sp.	*A. fumigatus* *Candida* sp. T. meganii	*Cladophialophora* sp.	No growth	*Cladophialophora* sp. *Epicoccum* sp. A. fumigatus
F15	Not born	*No growth*	A. niger *Penicillium* sp.	*A. versicolor* *T. ajello*	No growth	*Alternaria* sp. *A. fumigatus* *Cladophialophora* spp. x2 *Helminthosporium* sp.	*A. fumigatus* *Absidia* sp.
F16	Not born	No growth	No growth	T. ajello	No growth	No growth	*Cladophialophora* spp. x2
F17	No growth	No growth	*Wangiella werneckii*	Alternaria *sp.*	No growth	Not done	*Cladophialophora* sp. *Epicoccum* sp. T. rubrum
F18	*No growth*	No growth	*Cladosporium* sp. A. niger	*Absidia* sp. *Monosporium* sp. *Mucor* sp.	*Cladosporium* sp. T. ajello *Candida* sp.	Rhizopus *sp.*	*A. flavus* *A. glaucus* *A. versicolor* *Curvularia* sp. *Fusarium* sp.
F19	Not born	A. flavus	A. flavus	No growth	*Alternaria* sp.	*Alternaria* sp. *Cladophialophora* sp.	*Rhizopus* sp.
F20	Not born	*A. niger* *Mucor* sp.	*Mucor* sp. *Rhizopus* sp.	No growth	*A. niger* T. ajello	*Cladophialophora* sp.	*Cladophialophora* sp.
F22	Not born	No growth	No growth	Candida *sp.* *Penicillium* sp. *Alternaria* sp. *Cladophialophora* sp.	*Rhodotorula* sp. *Candida* sp.	Not done	*A. fumigatus* *Humicola* sp. *Penicillium* sp. *Trichosporon* sp.
F23	No growth	A. fumigatus A. glaucus	No growth	*A. fumigatus* *A. terreus* *Chysosporium* sp.	Not done	*Alternaria* sp. *Cladosporium* sp.	*Alternaria* sp. *A. flavus* *A. fumigatus* *Cladophialophora* spp. x2
F24	Not born	No growth	Not done	*Rhodotorula* sp. W. werneckii *Geotrichium* sp.	*A. glaucus*	Not done	*Alternaria* sp. *Cladophialophora* sp.
F25	Not born	No growth	Not done	*Cladophialophora* sp.	*Cladophialophora* sp.	Rhizopus *sp.*	*Alternaria* sp. *Cladophialophora* sp.

Foal	21/09 93	26/10/1993	23/11/93	24/12/93	20/01/94	24/02/94	29/03/94
F26	*Mucor* sp *A. flavus*	*Mucor* sp. *Rhizopus* sp.	*Alternaria* sp. *Rhizopus* sp	*Alternaria* sp. *Cladophialophora* sp. T. equinum	*Cladophialophora* sp. *Curvularia* sp. *Mucor* sp.	Alternaria *sp.* *A. terreus* *A. versicolor*	*A. fumigatus* *A. glaucus* *Cladophialophora* sp. *Fusarium* sp. *Penicillium* sp.
F29	Not born	A. glaucus *Penicillium* sp.	*Curvularia* sp	*Rhizopus* sp.	Not done	*Cladophialophora* spp. x2	No growth
F30	Not born	Not born	Not born	*Alternaria* sp. *Curvularia* sp	No growth	*Cladophialophora* sp. *Fusarium* sp.	*A. terreus* *Chrysoporium* sp. *Cladophialophora* sp. *Fusarium* sp.
F31	Not born	Alternaria *sp.* A. glaucus	*Monosporium* sp.	T. ajello *Cladosporium* sp. *Rhizopus* sp.	*Alternaria* sp. *A. niger* *Cladophialophora* sp. T. equinum	*Alternaria* sp. A. glaucus *Cladophialophora* sp.	*Cladophialophora* sp.
F32	Not born	*A. glaucus*	*Cladophialophora* sp. A. flavus	*Alternaria* sp	*Alternaria* sp. *Cladophialophora* sp. *Drechslera* sp.	No growth	*Alternaria* sp. *Cladophialophora* sp. *Penicillium* sp.
F33	Not born	Not born	No growth	A. glaucus *Rhizopus* sp.	No growth	No growth	*Cladophialophora* sp. *Monosporium* sp.
F34	Not born	Natal	Natal	Natal	No growth	No growth	*Exophiala* sp. *Fusarium* sp.
F35	Not born	Western Cape	Western Cape	Western Cape	*Alternaria* sp.	Not done	Not done
F37	Not born	*Chrysoporium* sp. *Monosporium* sp. *Mucor* sp.	A. niger *Penicillium* sp.		*Alternaria* sp. *Candida* sp.	*Alternaria* sp. *Cladosporium* sp. *Curvularia* sp.	*Cladophialophora* sp.
F38	Not born	Not born	Not born	No growth	No growth	A. fumigatus *Trichosporon* sp.	A. versicolor *A. glaucus* *Candida* sp.
F39	Not born	*Candida* sp. *Cladophialophora* sp. *Synecephalstrum* sp.	No growth	*Alternaria* sp	*Cladophialophora* sp.	*Absidia* sp. *Alternaria* sp. *A. flavus* *Cladophialophora* sp..	*Alternaria* sp. *Cladophialophora* sp. *Fusarium* sp.

Foal	02/04/94	4/05/94	2/6/94	27/6/94	2/8/94
F1	No growth	*Cladophialophora* sp. A. niger	A. terreus A. glaucus *Absidia* sp.	Scopulariopsis sp. *Monosporium* sp. *Curvularia* sp.	A. terreus *Scopulariopsis* sp. *Torula* sp. *Arthobotrys oligospora*
F2	No growth	*Fusarium* sp. *Epicoccum* sp. *Bipolaris* sp. A. versicolor	*Cladophialophora* sp. *Alternaria* sp.	*Absidia* sp.	*A. oligospora* A. flavus
F3	No growth	A. flavus	A. fumigatus *Beauveria* sp. A. terreus *Rhizopus* sp.	*Scopulariopsis* sp. *A. terreus*	*Cladophialophora* sp. A. flavus *Trichosporon* sp. *Chrysosporium* sp.
F5	*Cladophialophora* sp. *Penicillium* sp.	A. niger	*Botrytis* sp. *Candida* sp. *Chrysosporium* sp.	A. niger	A. terreus *Absidia* sp.
F6	*Rhizopus* sp.	*Penicillium* sp. *Cladophialophora* sp..	*Cladophialophora* sp. *Botrytis* sp. *Scopulariopsis* sp.	*Rhizopus* sp.	No growth
F8	*Cladophialophora* sp.	*Absidia* sp.	A. terreus *Cladophialophora* sp.	*Cladophialophora* sp. A. fumigatus	A. terreus Cladophialophora *sp.*
F9	No growth	A, fumigatus *A. terreus*	*Absidia* sp. A. fumigatus	*Alternaria* sp. A. fumigatus A. versicolor A. terreus Mucor *sp.*	A. terreus
F11	*Cladophialophora* sp. *Mucor* sp.	*Alternaria* sp. *Scopulariopsis* sp.	*A. terreus*	*Beauveria* sp. *Alternaria* sp. *Cladophialophora* sp. *Rhizopus* sp.	*Epicoccum* sp.
F13	*Cladophialophora* sp.	*Alternaria* sp.	*Cladophialophora* sp. *Alternaria* sp.	*Absidia* sp. *Cladophialophora* sp.	*Cladophialophora* sp. A. glaucus *Chrysosporium* sp. *Trichosporon* sp
F15	*Fusarium* sp. A. fumigatus *Absidia* sp.	A. niger *Peniciilium* sp.	*No growth*	A. terreus *Curvularia* sp. A. fumigatus	*Alternaria* sp. *Scopulariopsis* sp. *Fusarium* sp. *Drechslera* sp.

Foal	02/04/94	4/05/94	2/6/94	27/6/94	2/8/94
F16	*Cladophialophora* sp. *Fusarium* sp. *Chrysosporium* sp. *Alternaria* sp. *Humicola* sp.	A. glaucus A. terreus *Cladophialophora* sp. *Chrysosporium* sp.	No growth	*Absidia* sp. *Candida* sp.	A. terreus M. gypseum
F17	*Fusarium* sp.	No growth	A. versicolor *Alternaria* sp. A. terreus *Rhizopus* sp.	*Penicillium* sp.	*Absidia* sp.
F18	Cladophialophora sp. *Fusarium* sp. *Candida* sp.	A. niger *Fusarium* sp. A. fumigatus *Penicillium* sp.	*Penicillium* sp. *Fusarium* sp. *Alternaria* sp. *Absidia* sp.	*Absidia* sp. *Cladophialophora* sp. *Penicillium* sp.	*Cladophialophora* sp. *Verticillium* sp. *Trichosporum* sp.
F19	*Alternaria* sp.	*T. ajello* *Penicillium* sp.	*Cladophialophora* sp. *Alternaria* sp. *Penicillium* sp.	*Cladophialophora* sp. *Penicillium* sp.	*Cladophialophora* sp. *Verticillium* sp. A. glaucus *A. terreus*
F20	*Penicillium* sp. A. fumigatus A. versicolor Candida *sp.*	A. niger *T. ajello* A. niger *Penicillium* sp.	*Rhizopus* sp.	A. glaucus *Alternaria* sp. *Fusarium* sp.	*Alternaria* sp.
F22	*Cladophialophora* sp. A. fumigatus *Alternaria* sp. *Mucor* sp *Candida* sp. *W. werneckii*	*Cladophialophora* sp. A. terreus A. fumigatus *Alternaria* sp. *Candida* sp.	*Rhizopus* sp. A. flavus	A. fumigatus *Sepedonium* sp.	*Trichosporum* sp. *Humicola* sp. *Sepedonium* sp. A. glaucus *Cladophialophora* sp.
F23	*Rhizopus* sp.	*Cladophialophora* sp.	A. fumigatus T. equinum *Rhizopus* sp.	Alternaria *sp.*	A. terreus *Exophiala* sp.
F24	*Trichosporon* sp. A. fumigatus	No growth	No growth	*Cladophialophora* sp. *Absidia* sp.	*Alternaria* sp.
F25	A. fumigatus *Candida* sp. *Fusarium* sp.	*Penicillium* sp. *Absidia* sp.	*Cladophialophora* sp. *Fusarium* sp A. terreus *Rhizopus* sp.	*Fusarium* sp. *Alternaria* sp.	*Trichosporon* sp. *Humicola* sp. *Scopulariopsis* sp. *Rhinodocladiella* sp.
F26	*Candida* sp. *Chrysosporium* sp. A. fumigatus *Cladophialophora* sp.	*Alternaria* sp. *Absidia* sp. A. terreus	A. terreus *Penicillium* sp. *Absidia* sp.	*Penicillium* sp.	*Rhinododiella* sp. *Cladophialophora* sp.

Foal	02/04/94	4/05/94	2/6/94	27/6/94	2/8/94
F29	No growth	A. terreus	A. fumigatus Exophiala *sp.*	*Cladophialophora* sp. *Absidia* sp. *Botrytis* sp.	Beauveria sp A. terreus.
F30	*Penicillium* sp. A. glaucus A. terreus	A. glaucus *Cladophialophora* sp.	A. terreus *Fonsecaea* sp. *Mucor* sp.	*Alternaria* sp. *Absidia* sp.	*Alternaria* sp.
F31	Not done	No growth	A. terreus *A. fumigatus* *Alternaria* sp.	A. flavus *A. fumigatus* *Cladophialophora* sp.	Not done
F32	*Alternaria* sp.	*Penicillium* sp.	*Penicillium* sp. *Alternaria* sp. *Drechslera* sp. *Absidia* sp.	*Penicillium* sp.	A. terreus
F33	*A. glaucus* *A. fumigatus* *Monosporiium* sp. *Penicillium* sp.	*Mucor* sp. *Cladophialophora* sp.	Rhizopus *sp.* *Cladophialophora* sp.	*Fusarium* sp. A. flavus	*Cladophialophora* sp. A. flavus *Trichosporon* sp. *Chaetomonium* sp. *Torula* sp.
F34	*Absidia* sp. *Rhodotorula* sp. *Cladosporium* sp.	A. terreus *Cladophialophora* sp. *Absidia sp.*	*Absidia* sp. *Candida* sp.	A. flavus *Cladophialophora* sp. *Rhizopus* sp.	A. terreus
F35	A. terreus *A. versicolor*	*Bipolaris* sp. T. rubrum *Geotrichium* sp.	*Absidia* sp. *Cladophialophora* sp.	Cladophialophora *sp.* *Absidia* sp. *Penicillium* sp	*Rhinodocladiella* sp. *Fusarium* sp. A. fumigatus
F37	A. fumigatus *Alternaria* sp. *Chrysosporium* sp.	A. fumigatus	*Rhizopus* sp. *Scopulariopsis* sp.	*Absidia* sp.	*Epicoccum* sp. Rhizopus *sp.* *Fusarium* sp.
F38	Not done	*Geotrichium* sp.	*Cladophialophora* sp. *Rhizopus* sp. *Drechslera* sp	No growth	*Absidia* sp.
F39	*Rhizopus* sp.	*Chrysosporium* sp.	*Epicoccum* sp. *Alternaria* sp. *Helminosporium* sp.	*Cladophialophora* sp.	A. terreus *Absidia* sp. *Alternaria* sp.

APPENDIX V: INDIVIDUAL ANTIBODY LEVELS TO EACH VIRUS TESTED

Table 14: Neutralizing antibodies in 30 foals against EHV-1.

				Age in months of foal					Date of sampling							
Foal number	**Mare**	**Pre-colostrum**	**Post-colostrum**	**1**	**2**	**3**	**4**	**5**	**94/2/24**	**94/3/29**	**94/5/4**	**94/6/2**	**94/6/27**	**94/8/2**	**94/9/8**	**94/10/13**
1	80	80	120	80	30	ND	ND	0	0	0	ND	ND	10	15	10	15
2	60	0	40	20	0	0	0	0	0	0	0	0	0	0	30	20
3	20	0	20	15	10	0	0	0	0	0	0	0	20	0	0	10
5	20	ND	ND	0	0	0	0	0	0	0	0	0	0	5	0	0
8	40	0	40	5	0	0	0	0	0	0	0	0	0	0	0	0
6	20	ND	0	ND	0	15	0	0	0	0	0	0	0	0	0	0
9	60	10	30	0	0	0	0	0	0	0	0	0	0	0	0	0
11	15	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0
15	20	ND	ND	0	0	0	0	0	0	0	0	0	10	0	0	15
16	15	ND	20	0	0	0	0	0	0	0	0	0	0	0	0	15
17	15	0	30	0	0	0	0	0	0	0	0	0	0	15	0	10
18	20	0	20	0	15	0	0	0	0	0	0	0	5	0	0	5
19	10	0	15	5	0	0	0	0	0	0	0	0	10	0	0	0
20	40	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
22	15	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
23	30	0	20	0	0	0	0	0	0	0	0	10	5	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	20	10	0	0	0
25	60	0	15	10	0	0	0	0	0	0	0	0	15	0	15	0
26	10	0	0	0	0	0	0	0	0	0	0	0	0	40	40	10
29	120	ND	120	30	20	0	0	0	0	0	0	0	30	15	0	0
30	15	0	20	10	0	0	0	0	0	0	0	0	10	10	0	0
31	30	0	40	30	0	0	0	0	0	0	0	0	40	0	0	
32	30	20	120	60	60	20	0	ND	ND	0	0	0	40	0	0	0
33	60	ND	20	20	ND	0	ND	0	0	0	0	0	30	30	10	10
34	10	ND	15	0	0	0	0	0	0	0	0	0	0	0	10	0
35	40	ND	40	15	0	0	0	0	0	0	0	30	30	40	60	30
37	0	0	30	15	0	0	0	0	0	0	0	0	15	60	60	15
38	40	ND	60	60	30	15	0	0	0	0	0	0	0	20	0	0
39	40	ND	20	30	10	0	0	0	0	0	0	0	0	0	0	0
Average	**31.13**	**5.50**	**31.38**	**13.83**	**6.17**	**1.67**	**0**	**0**	**0**	**0**	**0**	**2.00**	**9.84**	**8.33**	**7.83**	**5.35**

Table 15: Complement-fixing antibodies in 30 foals against EHV-1.

				Age in months of foal					Sampling dates							
Foal number	**Mare**	**Pre-colostrum**	**Post-colostrum**	**1**	**2**	**3**	**4**	**5**	**94/2/24**	**94/3/29**	**94/5/4**	**94/6/2**	**94/6/27**	**94/8/2**	**94/9/8**	**94/10/13**
1	40	80	160	80	20	ND	ND	0	0	0	ND	ND	30	20	30	40
2	160	0	120	20	10	0	0	0	0	0	10	80	40	80	60	40
3	20	0	80	15	0	0	0	20	0	20	20	40	80	40	40	80
5	20	ND	ND	0	0	0	0	0	0	0	60	80	60	40	20	20
6	120	ND	160	160	40	0	0	0	0	0	0	20	30	15	10	40
8	80	0	160	15	10	0	0	0	0	0	30	40	30	40	20	40
9	20	0	20	10	10	10	0	0	0	0	0	40	30	30	20	40
11	30	ND	20	0	0	0	0	0	0	0	0	0	15	30	20	40
13	0	0	0	0	0	0	0	0	0	0	10	0	10	20	0	20
15	15	ND	ND	10	0	0	0	0	0	0	20	20	30	40	0	80
16	0	ND	20	0	0	0	0	0	0	0	10	0	10	20	0	80
17	10	0	10	10	0	0	0	0	0	0	10	0	60	10	20	160
18	20	0	0	0	10	0	0	0	0	0	20	40	15	15	20	80
19	60	0	80	0	0	0	15	0	0	0	15	20	15	0	20	20
20	20	0	40	40	0	0	0	0	0	0	15	20	15	15	0	40
22	40	0	40	0	0	0	0	0	0	0	15	20	15	0	0	80
23	0	0	0	0	0	0	0	0	0	0	15	20	15	10	10	40
24	20	0	20	0	0	10	0	0	0	0	0	20	20	10	0	80
25	160	0	160	160	20	10	0	0	0	0	60	40	15	20	0	40
26	40	0	0	80	10	0	0	0	0	0	0	20	10	15	20	80
29	40	ND	40	15	0	0	0	0	0	0	0	20	60	30	0	40
30	0	0	20	0	0	0	0	10	0	10	10	0	10	10	0	40
31	40	0	10	0	0	0	0	0	0	0	15	15	20	15	0	40
32	60	80	360	80	20	5	0	ND	ND	0	0	0	20	15	20	0
33	240	ND	240	240	ND	0	ND	0	0	0	0	20	15	20	0	40
34	40	ND	30	30	0	0	10	0	0	10	0	0	15	15	20	0
35	160	ND	240	160	0	0	0	10	0	10	10	40	1280	60	40	0
37	0	0	40	10	0	0	0	0	0	0	15	40	15	15	40	40
38	40	ND	80	80	20	0	0	0	0	0	15	0	10	15	0	0
39	60	ND	30	40	5	0	0	0	0	0	15	0	15	30	0	15
Average	**50.83**	**0**	**78.57**	**41.83**	**6.03**	**1.21**	**0.93**	**1.38**	**0**	**1.67**	**13.45**	**22.59**	**66.83**	**23.17**	**14.33**	**45.17**

Table 16: Neutralizing antibodies in 30 foals against EHV-4.

				Age in months of foal					Sampling dates							
Foal number	**Mare**	**Pre-colostrum**	**Post-colostrum**	**1**	**2**	**3**	**4**	**5**	**94/2/24**	**94/3/29**	**94/5/4**	**94/6/2**	**94/6/27**	**94/8/2**	**94/9/8**	**94/10/13**
1	40	60	160	160	40	ND	ND	0	0	0	ND	ND	0	20	30	60
2	80	0	40	30	10	0	0	0	0	0	0	0	0	30	0	0
3	60	0	40	15	0	0	0	0	0	0	0	0	20	15	0	20
5	60	ND	ND	0	0	0	0	0	0	0	0	0	0	20	0	0
6	40	ND	20	20	20	0	0	0	0	0	0	0	0	20	0	0
8	80	0	20	0	0	0	0	0	0	0	0	0	0	10	0	0
9	60	0	40	10	0	0	0	0	0	0	0	0	0	60	0	0
11	40	0	20	0	0	0	0	0	0	0	0	0	0	15	10	0
13	15	0	0	10	0	0	0	0	0	0	0	0	0	60	0	0
15	60	ND	ND	0	0	0	0	0	0	0	0	0	0	40	0	20
16	80	ND	30	20	10	0	0	0	0	0	0	0	0	0	0	40
17	30	0	20	0	0	0	0	0	0	0	0	0	60	80	0	0
18	20	0	40	10	10	0	0	0	0	0	0	0	10	10	0	0
19	40	0	30	15	0	0	0	0	0	0	0	0	0	0	0	0
20	30	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0
22	40	0	15	0	0	0	0	0	0	0	0	0	0	80	20	40
23	40	0	40	20	0	0	0	0	0	0	0	40	30	0	15	20
24	0	0	0	0	0	0	0	0	0	0	0	0	60	10	0	20
25	20	0	15	0	0	0	0	0	0	0	0	15	30	0	15	60
26	0	0	0	0	0	0	0	0	0	0	10	15	40	15	160	60
29	160	ND	120	80	20	0	0	0	0	0	0	0	80	30	15	15
30	40	0	30	15	0	0	0	0	0	0	0	0	10	10	10	10
31	40	0	40	30	15	0	0	0	0	0	0	60	120	20	15	10
32	60	40	80	80	20	10	0	ND	0	0	0	10	30	40	0	0
33	60	ND	40	40	ND	0	ND	0	0	0	15	0	80	120	20	20
34	80	ND	80	30	0	0	0	0	0	0	0	0	20	20	10	20
35	60	ND	30	30	0	0	0	0	0	0	0	40	30	20	60	20
37	0	0	30	15	0	0	0	0	0	0	0	0	20	60	60	60
38	0	ND	120	120	20	0	0	0	0	0	0	20	30	30	15	20
39	30	ND	30	30	10	0	0	0	0	0	0	15	30	0	0	15
Average	**45.50**	**5.00**	**40.89**	**26.00**	**6.03**	**0.35**	**0**	**0**	**0**	**0**	**0.86**	**7.41**	**23.33**	**27.83**	**15.17**	**17.67**

Table 17: Complement-fixing antibodies in 30 foals against EHV-4.

Foal number	Mare	Pre-colostrum	Post-colostrum	Age in months of foal					Sampling date							
				1	2	3	4	5	94/2/24	94/3/29	94/5/4	94/6/2	94/6/27	94/8/2	94/9/8	94/10/13
1	160	60	160	40	20	ND	ND	0	0	0	ND	ND	10	80	60	30
2	20	0	30	30	15	10	0	0	0	0	30	60	30	160	0	10
3	40	0	28	7	10	0	0	0	0	0	30	20	80	10	80	240
5	5	ND	ND	0	0	0	10	0	0	10	30	10	80	20	0	5
6	120	ND	120	120	80	15	0	0	0	0	20	40	30	10	10	5
8	60	0	30	0	0	0	0	0	0	0	60	40	30	30	60	20
9	160	0	60	40	20	10	0	0	0	0	160	40	30	30	15	15
11	160	0	60	40	20	10	0	0	0	0	160	40	30	30	15	15
13	15	0	0	10	10	0	15	10	0	0	60	15	30	40	10	10
15	15	ND	ND	0	0	0	0	0	0	0	30	15	80	15	0	10
16	0	ND	15	0	0	0	0	0	0	0	0	30	0	30	0	160
17	10	0	0	0	0	0	0	0	0	0	30	15	40	15	0	80
18	20	0	10	10	10	0	10	0	0	15	120	40	80	15	40	30
19	40	0	40	20	10	0	0	0	0	0	40	20	30	10	40	5
20	30	0	30	30	0	0	0	0	0	10	20	60	60	0	5	0
22	30	0	20	0	0	0	0	0	0	0	15	30	40	0	0	10
23	10	0	0	0	0	0	0	0	0	0	20	20	40	15	0	10
24	0	0	10	0	0	0	0	0	0	0	20	30	60	15	10	10
25	40	0	30	30	10	0	0	0	0	0	90	80	160	60	0	0
26	60	0	0	0	0	0	5	0	0	0	60	20	30	15	80	15
29	60	ND	60	40	15	10	10	0	0	0	20	30	640	60	30	20
30	30	0	10	10	0	0	0	40	10	0	40	10	10	20	0	0
31	10	0	0	10	10	10	0	0	0	0	60	60	80	20	5	0
32	80	20	140	140	15	10	0	ND	0	0	80	240	160	10	0	0
33	80	ND	28	20	ND	20	ND	0	0	0	0	30	120	20	0	0
34	60	ND	60	20	0	0	15	10	0	0	15	10	20	10	40	0
35	40	ND	15	15	0	0	60	15	60	15	40	140	140	120	40	28
37	40	0	15	0	15	10	0	0	0	0	15	140	60	30	60	30
38	60	ND	60	60	10	0	0	0	0	0	0	10	15	30	0	0
39	30	ND	30	30	0	10	0	0	0	0	30	40	80	1280	0	20
Average	**48.50**	**0**	**37.89**	**24.07**	**9.31**	**3.97**	**4.46**	**2.59**	**2.33**	**1.67**	**44.70**	**46.03**	**76.50**	**73.33**	**20.00**	**25.93**

Table 18: Neutralizing antibodies in 30 foals against ERV-1.

Foal number	Mare	Pre-colostrum	Post-colostrum	Age in months of foal						Sampling date						
				1	2	3	4	5	6	94/3/29	94/5/4	94/6/2	94/6/27	94/8/2	94/9/8	94/10/13
1	40	320	320	320	320	ND	ND	20	0	0	ND	ND	0	0	0	0
2	20	0	160	160	160	160	80	10	0	0	0	0	0	0	0	0
3	320	0	320	ND	ND	80	40	0	0	0	0	0	0	0	0	0
5	320	ND	ND	160	80	40	0	0	0	0	0	0	0	0	0	0
6	80	ND	60	60	20	0	0	0	0	0	0	0	0	0	0	0
8	120	20	240	40	15	10	0	0	0	0	0	0	0	0	0	0
9	80	0	60	10	0	0	0	0	0	0	0	0	0	0	0	0
11	160	ND	120	20	10	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	60	ND	ND	160	40	10	0	0	0	0	0	0	0	0	0	0
16	320	ND	320	ND	160	80	40	0	0	0	0	0	0	0	0	0
17	80	20	80	80	80	10	0	0	0	0	0	0	0	0	0	0
18	160	0	320	120	60	20	0	0	0	0	0	0	0	0	0	0
19	320	0	320	320	240	60	20	10	0	0	0	0	0	0	0	0
20	120	0	80	10	5	0	0	0	0	0	0	0	0	0	0	0
22	60	0	40	15	10	0	0	0	0	0	0	0	0	0	0	0
23	60	0	80	20	10	0	0	0	0	0	0	0	0	0	0	0
24	40	0	80	10	10	0	0	0	0	0	0	0	0	0	0	0
25	320	0	320	160	160	0	0	0	0	0	0	0	0	0	0	0
26	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	320	ND	120	80	40	10	0	0	0	0	0	0	0	0	0	0
30	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	120	0	80	40	15	0	0	0	0	0	0	0	0	0	0	0
32	120	15	40	40	20	0	0	ND	0	0	0	0	0	0	0	0
33	120	ND	20	40	ND	0	ND	0	0	0	0	0	0	0	0	0
34	240	ND	240	240	10	0	0	0	0	0	0	0	0	0	0	0
35	120	ND	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	320	0	80	40	10	5	0	0	0	0	0	0	0	0	0	0
38	240	ND	240	240	160	15	10	0	0	0	0	0	0	0	0	0
39	120	ND	80	40	10	0	0	0	0	0	0	0	0	0	0	0
Average	**149.3**	**0**	**136.40**	**86.60**	**58.80**	**17.24**	**6.79**	**1.38**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 19: Neutralizing antibodies in 30 foals against ERV-2.

Foal number	Mare	Pre-colostrum	Post-colostrum	Age in months of foal					Sampling date							
				1	2	3	4	5	94/2/24	94/3/29	94/5/4	94/6/2	94/6/27	94/8/2	94/9/8	94/10/13
1	20	40	80	40	20	ND	ND	0	0	0	ND	ND	0	0	10	10
2	60	0	20	15	5	0	0	0	0	0	0	0	0	0	10	10
3	20	0	40	15	5	0	0	0	0	0	0	10	15	30	30	20
5	0	ND	ND	40	20	10	0	0	0	0	0	0	0	20	0	10
6	30	ND	10	ND	10	40	20	15	15	15	20	20	10	0	30	40
8	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	10	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
11	15	ND	20	0	0	0	0	0	0	0	0	0	0	5	10	0
13	0	0	0	0	0	15	10	0	0	0	0	0	5	0	0	0
15	0	ND	ND	ND	0	0	0	0	0	0	0	10	10	20	20	10
16	0	ND	20	ND	10	10	0	0	0	0	0	0	0	0	0	0
17	80	60	80	40	20	0	0	0	0	0	0	0	0	0	0	15
18	40	0	30	10	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
20	20	0	0	0	0	0	0	0	0	0	0	0	0	0	30	20
22	15	0	0	0	0	0	0	0	0	0	0	15	30	15	20	20
23	10	0	0	0	0	0	0	0	0	0	0	10	10	0	0	5
24	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10	15
25	20	0	20	10	0	0	0	0	0	0	0	0	0	0	0	0
26	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	60	ND	15	0	0	0	0	0	0	0	0	0	30	10	10	10
30	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	20	0	0	0	0	0	0	0	20	60	0	10	15	30
32	10	10	15	20	15	0	0	ND	ND	0	0	0	30	10	0	10
33	120	ND	30	80	ND	10	ND	10	ND	10	0	60	30	30	30	20
34	20	ND	10	10	0	0	0	0	0	0	0	0	0	0	15	0
35	0	ND	0	0	0	0	0	0	0	0	0	0	0	0	15	15
37	60	0	30	15	0	0	0	0	0	0	0	0	0	0	0	0
38	20	ND	20	20	0	0	0	0	0	0	0	0	0	30	10	30
39	60	ND	30	20	15	0	0	0	0	0	0	0	0	0	0	0
Average	**24.83**	**0**	**18.57**	**12.41**	**4.14**	**2.93**	**1.07**	**0.86**	**0.54**	**0.833**	**1.38**	**6.38**	**5.67**	**6.00**	**8.83**	**9.67**

Table 20: Neutralizing antibodies in 30 foals against EAdV.

Foal number	Mare	Pre-colostrum	Post-colostrum	Age in months of foal					Sampling date							
				1	2	3	4	5	94/2/24	94/3/29	94/5/4	94/6/2	94/6/27	94/8/2	94/9/8	94/10/13
1	240	0	320	160	120	ND	ND	0	0	0	ND	ND	60	30	30	160
2	160	0	160	160	60	20	15	0	0	20	10	0	0	20	10	20
3	0	80	80	30	0	0	10	0	0	0	0	0	40	0	30	60
5	240	ND	ND	240	160	40	15	0	0	0	0	0	0	0	0	20
6	60	ND	15	15	60	60	80	60	80	60	30	0	10	10	80	320
8	120	0	60	60	10	0	0	0	0	0	0	0	0	10	20	10
9	240	0	320	120	30	30	0	0	0	0	0	15	15	10	0	0
11	240	ND	160	120	40	30	10	0	0	0	0	0	0	0	0	160
13	80	0	80	80	60	60	40	40	40	0	0	0	0	0	0	80
16	120	ND	320	320	160	120	30	15	30	15	10	40	30	10	160	80
17	320	60	320	80	20	10	0	80	80	40	20	20	0	0	0	30
18	40	0	60	10	30	0	0	15	15	10	320	80	60	0	15	10
19	80	0	60	30	30	30	0	0	0	0	0	30	15	0	0	0
20	20	0	15	10	20	10	10	20	20	0	0	80	120	0	20	240
22	40	0	60	20	10	0	0	0	0	60	40	10	0	30	0	0
23	240	0	160	60	20	15	0	0	0	0	30	20	15	10	0	80
24	20	0	0	10	20	0	0	0	0	0	40	80	40	15	10	120
25	320	0	240	30	30	0	0	0	0	0	30	15	0	30	0	30
26	40	0	0	0	0	0	0	0	0	10	20	40	80	40	20	80
29	120	ND	120	40	30	10	0	0	0	30	30	0	10	0	40	40
30	30	0	30	30	15	0	0	0	15	0	0	30	0	0	0	60
31	120	0	240	120	80	30	15	10	15	10	120	60	40	20	15	20
32	120	120	160	160	120	20	15	ND	0	0	0	20	15	10	0	0
33	60	ND	20	40	ND	0	ND	0	0	0	0	0	30	20	10	40
34	120	ND	320	320	0	0	20	30	0	0	20	30	20	0	0	20
35	240	ND	120	120	0	0	0	0	0	0	0	0	30	0	0	0
37	120	0	80	40	20	30	0	0	0	0	0	120	15	60	30	40
38	120	ND	240	240	160	40	30	0	0	0	0	0	40	15	10	30
39	240	ND	160	160	160	30	0	0	0	0	0	0	20	40	10	0
Average	**132.30**	**0**	**140.00**	**96.17**	**51.55**	**20.69**	**10.40**	**9.31**	**9.83**	**8.50**	**24.83**	**23.79**	**23.50**	**12.67**	**17.00**	**59.67**

Table 21: Haemagglutinating antibodies in 30 foals against EIV.

Foal number	Mare	Pre-colostrum	Post-colostrum	Age in months of foal							Sampling date								
				1	2	3	4	5	6	7	94/2/24	94/3/29	94/5/4	94/6/2	94/6/27	94/8/2	94/9/8	94/10/13	95/3/5
1	20	0	60	60	20	ND	ND	20	10	ND	20	10	ND	ND	0	0	120	0	10
2	120	0	640	240	160	80	10	80	15	10	80	15	10	0	0	0	40	5	80
3	60	0	60	40	10	10	0	20	0	0	20	0	0	20	40	0	ND	0	0
5	40	ND	ND	40	120	60	10	10	0	0	10	0	0	0	10	0	40	0	20
6	80	ND	120	120	60	10	10	0	0	0	0	0	0	0	20	40	0	0	20
8	240	0	120	120	120	10	10	10	10	10	10	10	10	60	320	40	0	0	40
9	320	0	640	640	320	160	120	20	15	15	20	15	15	10	15	0	10	0	80
11	640	ND	640	120	120	60	20	20	0	10	20	0	10	0	0	10	40	0	5
13	240	0	320	320	80	60	10	10	5	10	10	5	10	0	10	15	20	0	20
15	640	ND	320	320	320	10	10	20	10	0	20	10	0	40	10	0	0	0	20
16	240	ND	240	120	240	160	80	30	30	10	30	30	10	40	10	80	0	0	40
17	1280	0	1280	1280	240	80	19	10	30	15	10	30	15	0	0	20	40	20	80
18	640	0	1280	1280	160	80	15	20	30	5	20	30	5	15	15	40	0	80	160
19	240	0	240	160	160	80	10	10	15	5	10	15	5	15	0	0	0	0	40
20	1280	0	1280	1280	160	40	15	20	20	5	20	20	5	15	0	10	80	0	10
22	640	0	640	1280	640	160	10	10	15	5	10	15	5	0	20	0	40	0	80
23	320	0	240	240	240	80	20	20	0	10	20	0	10	0	0	10	40	0	80
24	320	0	640	120	320	10	10	0	0	15	0	0	15	10	10	15	40	5	80
25	640	0	640	320	120	80	10	10	20	10	10	20	10	15	10	15	40	0	0
26	240	0	0	160	120	20	20	10	0	0	10	0	0	10	0	10	40	0	80
29	320	ND	240	240	15	40	10	20	10	10	20	10	10	0	0	0	40	320	80
30	160	0	160	ND	ND	40	15	0	0	10	0	0	10	0	0	0	20	0	0
31	80	0	40	40	40	10	0	0	15	15	0	15	15	0	0	10	80	0	0
32	0	0	160	160	80	40	15	10	10	15	10	10	15	0	10	10	80	0	0
33	80	ND	80	80	ND	60	ND	10	5	0	10	5	0	0	15	80	0	0	80
34	80	ND	40	40	10	5	10	10	10	0	10	10	0	0	40	ND	ND	0	0
35	60	ND	80	80	0	0	5	15	5	20	15	5	20	15	120	0	ND	0	320
37	60	0	60	30	40	10	5	0	0	10	0	0	10	30	60	120	80	80	20
38	240	ND	320	240	40	10	5	5	5	0	5	5	0	60	240	40	ND	40	40
39	80	ND	120	120	120	30	30	20	0	0	20	0	0	15	0	30	160	0	80
Average	**313.33**	**0**	**372.14**	**320.3**	**146.0**	**51.60**	**18.00**	**14.67**	**9.50**	**7.41**	**14.67**	**9.50**	**7.41**	**12.80**	**32.50**	**20.52**	**40.38**	**18.33**	**51.17**

REFERENCES

AGIUS, C.T., CRABB, B.S., DRUMMER, H.E., REUBEL, G.H. & STUDDERT, M.J., 1994. Studies of the DNA, proteins and cellular associations of equine gammaherpesviruses 2 and 5. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 277-285.

ALLEN, G.P., 2002. Respiratory infections caused by equine herpesviruses types 1 and 4. *In*: Equine Respiratory Diseases. Lekeux, P., (ed),. International Veterinary Information Service. www.ivis.org.

ALLEN, G.P. & BRYANS, J.T., 1986. Molecular epizootiology, pathogenesis and prophylaxis of Equine herpesvirus-1 infections. *Progress in Veterinary Microbiology and Immunology,* 2, 78-144.

ALLEN, G.P., YEARGAN, M.R., TURTINEN, L.W., BRYANS, J.T. & McCOLLUM, W. H., 1983. Molecular epizootiologic studies of equine herpesvirus-1 infections by restriction endonuclease fingerprinting. *American Journal of Veterinary Research*, 44, 263-271.

ALLEN, G.P., YEARGAN, M.R. & COOGLE, L.D., 1988. Equid herpes virus glycoprotein 13 (gp13): Epitope analysis, gene structure and expression in *E. coli*. *In*: Equine Infectious Diseases V. Proceedings of the 5th International Conference, Lexington, Powell, D.G.(ed). University Press of Kentucky, 103-110.

ALLES, A.J., WALDRON, L.S., SIERRA, A.R. & MATTIA, A.R., 1995. Prospective comparison of direct immunofluorescence and conventional staining methods for detection of *Giardia* and *Cryptosporidium* spp in human fecal specimens. *Journal of Clinical Microbiology***,** 33**,** 1632-1634.

ÅNESTAD, M.G. & MAAGAARD, O., 1990. Rapid diagnosis of equine influenza. *The Veterinary Record*, 126, 550-551.

ANTAL, T., SZABO, I., ANTAL, V., VAJDA, G., POLNER, A., TOTTH, B., SZOLLAR, I. & STIPKOVITS, L., 1988. Respiratory disease of horses associated with mycoplasmal infection. *Journal of Veterinary Medicine*, 35, 264-270.

BAGUST, T.J., PASCOE, R.R. & HARDEN, T.J., 1972. Studies on equine herpesviruses 3. The incidence in Queensland of three different equine herpesvirus infections. *Australian Veterinary Journal*, 48, 47-53.

BAILEY, G.D. & LOVE, D.N., 1991. Oral associated bacterial infection in horses: Studies on the normal anaerobic flora from the pharyngeal surface and its association with lower respiratory tract and para-oral infections. *Veterinary Microbiology*, 26, 367-379.

BARTON, M.D. & HUGHES, K.L., 1980. *Corynebacterium equi*: a review. *The Veterinary Bulletin*, 50, 65-80.

BAXI, N.K., EFSTATHIOU, S., LAWRENCE, G., WALLEY, J.M., SLATER, J.D. & FIELD, H.J., 1995. The detection of latency-associated transcripts of equine herpesvirus 1 in ganglionic neurons. *Journal of General Virology*, 76, 113-118.

BAYLEY, W.M., REED, S.M., FOREMAN J.H., TRAUB, J.L. & McMURPHY, R.M., 1982. Equine bronchopneumonia due to *Bordetella bronchiseptica*. *Equine Practice*, 4, 25–32.

BERNARD, B., DUGAN, J., PIERCE, S. & GARDINER, I., 1991. The influence of foal pneumonia on future racing performance. *Proceedings of the American Association of Equine Practioners*. 17-18.

BINNS, M., McCANN, S., ZHANG, L., WOOD, J. & MUMFORD, J.A., 1994. Molecular epidemiology of EHV-1 and EHV-4: A search for variable restriction sites. *In:* Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 237–241.

BLUNDEN, A.S., HANNANT, D., LIVESAY, G. & MUMFORD, J.A., 1994. Susceptibility of ponies to infections with *Streptococcus pneumoniae* (capsule type 3). *Equine Veterinary Journal*, 26, 22-28.

BOGDAN, J. R., MORLEY, P. S., TOWNSEND, H. G. G. & HAINES, D. M., 1992. Effect of Influenza A/equine/H3N8 virus isolate variation on the measurement of equine antibody responses. *Canadian Journal of Veterinary Research*, 57, 126-130.

BORCHERS, K., FRÖLICH, K. & LUDWIG, H., 1999. Detection of equine herpesvirus types 2 and 5 (EHV-2 and EHV-5) in Przewalski's wild horses. *Archives of Virology*, 144, 771-780.

BORCHERS, K., WOLFINGER, U., GOLTZ, M., BROLL, H. & LUDWIG, H., 1997. Distribution and relevance of equine herpesvirus type 2 (EHV-2) infections. *Archives of Virology*, 142, 917-928.

BORCHERS, K., WOLFINGER, U., LAWRENZ, B., SCHELLENBACH, A. & LUDWIG, H., 1977. Equine herpesvirus 4 DNA in trigeminal ganglia of naturally infected horses detected by direct *in situ* PCR. *Journal of General Virology*, 78, 109-114.

BRAIN, J.D. & VALBERG, P.A., 1979. Deposition of aerosol in the respiratory tract. *American Reviews of Respiratory Disease*, 120, 1325-1373.

BREATHNACH, C.C., YEARGAN, M.R., SHEORAN, A.S. & ALLEN, G.P., 2001. The mucosal humoral immune response of the horse to infective challenge and vaccination with equine herpesvirus-1 antigens. *Equine Veterinary Journal*, 33, 651-657.

BROWNING, G.F., BULACH, D.M., FICORILLI, N., ROY, E.A., THORP, B.H. & STUDDERT, M.J., 1988. Latency of equine herpesvirus 4 (equine rhinopneumonitis virus). *The Veterinary Record*, 123, 518-519.

BROWNING, G.F. & STUDDERT, M.J., 1987. Genomic heterogeneity of equine betaherpesviruses. *Journal of General Virology*, 68, 1441-1447.

BRYANS, J.T., 1981. Application of management procedures and prophylactic immunization to the control of equine rhinopneumonitis. *In:* Proceedings of the 26th Annual Convention of the American Association for Equine Practioners, Anaheim, 1981. 259-272.

BRYANS, J.T. & ALLEN, G.P., 1989. Herpes viral diseases of the horse. *In:* Herpes Viral Diseases of Cattle, Horses and Pigs. Whittman G., (ed). Boston, Kluwer, 176.

BRYANS, J.T., DOLL, E.R., WILSON, J.C. & ZENT, W.W., 1967. Epizootiologic features of disease caused by *Myxovirus influenze* A equine. *American Journal of Veterinary Research*, 28, 9-17.

BURLESON, F.G., CHAMBERS, T.M. & WIEDBRAUK, D.L., 1992. Virology: A laboratory manual, Academic Press, Inc.

BÜRKI, F. & SIBALIN, M., 1972. Conclusions and questions arising from a study of serology and virology of equine influenza. *In:* Infectious Diseases III. Proceedings of the 3rd International Conference on Equine Infectious Diseases, Paris, Karger, Basel,

BURRELL, M.H., MACKINTOSH, M.E. & TAYLOR, C.E.D., 1996. Isolation of *Streptococcus pneumoniae* from the respiratory tract of horses. *Equine Veterinary Journal*, 18, 183-186.

BURRELL, M.H., MACKINTOSH, M.E., WHITWELL, K.E., MUMFORD, J.A. & ROSSDALE, P.D., 1985. A two year study of respiratory disease in a Newmarket stable: some preliminary observations. *In:* Proceedings of the Society of Veterinary Epidemiology and Preventative Medicine. 27th - 29th March 1985, 74-83.

BURRELL, M.H., WOOD, J.L.N., WHITWELL, K.E., CHANTER, N., MACKINTOSH, M.E. & MUMFORD, J.A., 1986. Respiratory disease in Thoroughbred horses in training: the relationships between disease and viruses, bacteria and environment. *The Veterinary Record*, 139, 308-313.

BURRELL, M.H., WHITWELL, K.E., WOOD, J.L. & MUMFORD, J.A., 1994. Respiratory disease in Thoroughbred horses in training: the relationships between disease and viruses, bacteria and environment. *The Veterinary Record*, 139, 308-313.

BURROWS, R. & GOODRIDGE, D., 1978. Observations of picornavirus, adenovirus and equine herpesvirus infections in the Pirbright pony herd. *In:* Equine Infectious Diseases IV. Proceedings of

the 4th International Conference on Equine Infectious Diseases, Lyon, 1976, Bryans, J. T. & Gerber, H., (eds). Veterinary Publications, Inc., 155-159.

BURROWS, R., GOODRIDGE, D., DENYER, M., HUTCHINGS, G. & FRANK, C.J., 1982. Equine influenza infections in Great Britain, 1979. *The Veterinary Record*, 110, 494-497.

CAMPBELL, M.C. & STEWART, J.L. (eds), 1981. The Medical Mycology Handbook, John Wiley and sons, New York, Chichester, Brisbane, Toronto.

CARTER, G.R. & COLE, J.R. (eds.), 1990. Diagnostic Procedures in Veterinary Bacteriology and Mycology, 5th edition. Academic Press Inc.

CAVANAGH, D., 1997. *Nidovirales*: A new order comprising *Cornonaviridae* and *Arteriviridae*. *Archives of Virology*, 142, 629-633.

CHALQUEST, R.R., 1962. Cultivation of the infectious-synovitis-type pleuropneumonia-like organisms. *Avian Diseases*, 6, 36-43.

CHAMBERS, T.M., HOLLAND, R.E., TUDOR, L.R., TOWNSEND, H.G.G., COOK, A., BOGDAN, J., LUNN, D.P., HUSSEY, S., WHITAKER-DOWLING, P., YOUNGNER, J.S., SEBRING, R.W., PENNER, S.J. & STIEGLER, G.L., 2001. A new modified live influenza virus vaccine: phenotypic stability, restricted spread and efficacy against heterologous virus challenge. *Equine Veterinary Journal*, 33, 630–636.

CHAMBERS, T.M., LAI, C.K., FRANKLIN, K.M. & POWELL, D.G., 1994. Recent evolution of the haemagglutinin of equine-2 influenza virus in the USA. *In:* Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, June 1994, Nakajima, H. & Plowright, W. (eds), 175-180.

CHANTER, N., 1998. Importance and aspects for control of streptococcal infections in the horse. *In*: Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O.-R. (eds), 76-81.

CHIRNSIDE, E.D., 1992. Equine arteritis virus: an overview. *British Veterinary Journal*, 148, 181-19.

CLABOUGH, D.L., 1991. Diagnosing viral infections. Eq*uine Veterinary Data*, 12, 250-251.

CLARKE, A.F., 1987a. A review of environmental and host factors in relation to equine respiratory disease. *Equine Veterinary Journal,* 19, 435-441.

CLARKE, A.F., 1987b. Chronic pulmonary disease. A multifaceted disease complex in the horse. *Irish Veterinary Journal,* 41, 258-264.

COETZER, J.A.W. & ERASMUS, B.J., 1994a. Chapter 44. African horsesickness. *In*: Infectious Diseases of Livestock with Special Reference to Southern Africa. Coetzer, J.A.W., Thomson, G.R. & Tustin, R.C. (eds), Oxford University Press, Cape Town. 460-475.

COETZER, J.A.W. & ERASMUS, B.J., 1994b. Chapter 45. Equine encephalosis. *In*: Infectious Diseases of Livestock with Special Reference to Southern Africa. Coetzer, J.A.W., Thomson, G.R. & Tustin, R.C. (eds), Oxford University Press, Cape Town. 476-479.

COHEN, N.D., 1994. Causes of and farm management factors associated with disease and death in foals. *Journal of the American Veterinary Medical Association*, 204, 1644-1651.

COLLINSON, P.N., O'REILLY, J.J., FICORILLI, N. & STUDDERT, M.J., 1994. Isolation of equine herpesvirus type 2 (equine gammaherpesvirus 2) from foals with keratoconjunctivitis. *Journal of the American Veterinary Medical Association*, 205, 329-331.

CONNOR, M., GILLESPIE, J., SCHIFF, E., HOLMES, D., FREY, M. & QUICK, S., 1985. Experimental infection of horses and ponies by oral and intranasal routes with New York State Reovirus type 3 and German Reovirus types 1 and 3. *Zentralblatt für Veterinärmedizin*, 707-717.

COOK, R.F., SINCLAIR, R. & MUMFORD, J.A., 1988. Detection of influenza nucleoprotein antigen in nasal secretions from horses infected with A/equine influenza (H3N8) viruses. *Journal of Virological Methods*, 20, 1-20.

CRABB, B.S., DRUMMER, H.E., REUBEL, G.H., McPHERSON, C.M., BROWNING, G.F. & STUDDERT, M.J., 1994. Poster abstract: A type-specific, serological test for the detection of antibodies to equid herpesviruses-4 and -1. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 349.

CRABB, B.S., MACPHERSON, C.M., REUBEL, G.H., BROWNING, G.F., STUDDERT, M.J. & DRUMMER, H.E., 1995. A type-specific serological test to distinguish antibodies to equine herpesviruses 4 and 1. *Archives in Virology*, 140, 45-258.

DALGLISH, R. A., 1992. The international movement of horses - the current disease situation. *In:* Proceedings of the 9th International Conference of Racing Analysts and Veterinarians, New Orleans, 1992, 37-53.

DEBOER, G.F. & OSTERHAUS, A.D.M.E., 1978. Prevalence of antibodies to equine viruses in The Netherlands. *In*: Equine Infectious Diseases IV. Proceedings of the 4th International Conference on Equine Infectious Diseases, Lyon, 1976, Princeton, New Jersey, Bryans, J.T. & Gerber, H. (eds). Veterinary Publications, 487-492.

DITCHFIELD, W.J., 1969. Rhinoviruses and parainfluenza viruses of horses. *Journal of the Veterinary Medical Association*, 155, 384-390.

DOLL, E.R. & BRYANS, J.T., 1962. Development of complement-fixing and virus-neutralising antibodies in viral rhinopneumonitis of horses. *American Journal of Veterinary Research*, 23, 843-846.

DOLL, E.R. & BRYANS, J.T., 1963. Epizootiology of equine viral rhinopneumonititis. *Journal of the American Veterinary Medical Association*, 142, 31-37.

DOLL, E.R., McCOLLUM, W.H., WALLACE, W.E., BRYANS, J.T. & RICHARDS, M.G., 1953. Complement-fixation reactions in equine virus abortion. *American Journal of Veterinary Research,* 40-45.

DUNOWSKA, M., MEERS, J. & WILKS, C., 1998. Poster: Equine respiratory viruses in New Zealand. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 538-539.

EDINGTON, N., BRIDGES, C.G. & PATEL, J.R., 1986. Endothelial cell infection and thrombosis and paralysis caused by equid herpesvirus-1: equine stroke. *Archives of Virology*, 90, 111-124.

EDINGTON, N. & BRIDGES, C.G., 1990. One way protection between equid herpesvirus 1 and 4 *in vivo*. *Research in Veterinary Science*, 48, 235-239.

EDINGTON, N., BRIDGES, C.G. & HUCKLE, A., 1985. Experimental reactivation of equid herpesvirus 1 (EHV 1) following the administration of corticosteroids. *Equine Veterinary Journal*, 17, 369-372.

EL HARRAK, M., HMIDOUCH, A., CHAKRI, A., OURAGH, L. & AKKARI, A., 1998. Poster: Epidemiology of A/equi-1 and A/equi-2 infections in Morocco. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 540 - 541.

ENGLAND, J.J., McCHESNEY, A.E. & CHOW, T.L., 1978. Isolation and identification of equine adenoviruses. *In:* Infectious Diseases IV. Proceedings of 4th International Conference on Equine Infectious Disease, Lyon 1976, Bryans, J. T. & Gerber, H. (eds). Veterinary Publications, Inc., 147-150.

ERASMUS, B.J., 1963. Equine viral rhinopneumonitis. *Journal of the South African Veterinary Medical Association*, 34, 461-469.

EVERMANN, J.F., WARD, A.C. & VAN DER SCHALIE, J., 1987. Equine Influenza outbreak 1986. *Journal of Equine Veterinary Science,* 7, 43-44.

FOLSOM, R.W., LITTLEFIELD-CHABAUD, M.A., FRENCH, D.D., POURCIAU, S.S., MISTRIC, L. & HOROHOV, D.W., 2001. Exercise alters the immune response to equine influenza virus and increases susceptibility to infection. *Equine Veterinary Journal*, 33, 661-669.

FRERICHS, C.C., FRERICHS, G.N. & BURROWS, R., 1973. Some aspects of the hemagglutination-inhibition test used in serological studies of equine influenza. *Symposia Series in Immunobiological Standardization*, 20, 338-346.

FREVERT, C. W., 1994. Respiratory distress in the foal: prematurity vs. sepsis. *Equine Practice*, 16, 9-12.

FU, Z.F., ROBINSON, A.J., HORNER, G.W., DICKENSON, L.G., GRIMMITT, J.B. & MARSHALL, R.B., 1986. Respiratory disease in foals and the epizootiology of equine herpesvirus type 2 infection. *New Zealand Veterinary Journal*, 21, 23-26.

FUKUNAGA, Y., IMAGAWA, H., TABUCHI, E. & AKIYAMA, Y., 1981. Clinical and virological findings on experimental equine arteritis in horses. *Bulletin of the Equine Research Institute,* Japan, 18, 110-118.

GALÁN, J.E. & TIMONEY, J.F., 1988. Immunologic and genetic comparison of *Streptococcus equi* isolates from the United States and Europe. *Journal of Clinical Microbiology*, 26, 1142-1146.

GALLAHAR, G.L., 1965. Isolation of *Bordetella bronchiseptica* from horses. *The Veterinary Record*, 77, 632-633.

GALOSI, C.M., NOSETTO, E.O., OLIVA, G.A., ECHEVERRIA, M.G., PECORARO, M.G., NORIMINE, J., TOHYA, Y., ETCHEVERRIGARAY, M.E. & TAKAHASHI, E., 1994. Poster abstract: Characterisation of equid herpesvirus-1 (EHV-1) isolated in Argentina from 1979 to 1991. *In:* Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W., (eds), 347.

GERBER, H., 1969. Clinical features, sequelae and epidemiology of equine influenza. *In*: Equine Infectious Diseases II. Proceedings of the 2nd International Conference. 63-80.

GIBSON, J.S., SLATER, J.D., AWAN, A.R. & FIELD, H.J., 1992. Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation. *Archives of Virology*, 123, 351-366.

GIGUÈRE, S. & PRESCOTT, J.F., 1998. Virulence mechanisms of, and immunity to, *Rhodococcus equi*: a review. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade, J.F., Mumford, J.A. & Kaaden, O-R. (eds),103-110.

GILBERT, S.A., TIMONEY, P.J., McCOLLUM, W.H. & DEREGT, D., 1997. Detection of equine arteritis virus in the semen of carrier stallions by using a sensitive-nested PCR assay. *Journal of Clinical Microbiology*, 35, 2181-2183.

GILKERSON, J., JORM, L.R., LOVE, D.N., LAWRENCE, G.L. & WHALLEY, J.M., 1994. Epidemiological investigation of equid herpesvirus-4 (EHV-4) excretion assessed by nasal swabs taken from Thoroughbred foals. *Veterinary Microbiology*, 39, 275-283.

GLASER, A.L., CHIRNSIDE, E.D., HORZINEK, M.C. & DE VRIES, A.A.F., 1997. Equine arteritis virus. *Theriogenology*, 47, 1275-1295.

GLEESON, L.J., STUDDERT, M.J. & SULLIVAN, N.D., 1978. Pathogenecity and immunologic studies of equine adenovirus in specific pathogen free foals. *American Journal of Veterinary Research*, 39, 1636-1642.

GOLNIK, W., MICHALASKA, Z. & MICHALAK, T., 1981. Natural equine viral arteritis in foals. *Schweizer Archiv für Tierheilkunde*,123, 523-533.

GUTHRIE, A.J., HOWELL, P.G., HEDGES, J.F., BOSMAN, A-M., BALASURIYA, U.B.R., McCOLLUM, W.H., TIMONEY, P.J. & MACLACHLAN, N.J., 2003. Lateral transmission of equine arteritis virus among Lipizzaner stallions in South Africa. *Equine Veterinary Journal*, 35, 596-600.

GUTHRIE, A.J., 2004. Personal communication.

HAMMER, C.J., BOOTH, J.A., ETZEL, L. & TYLER, H.D., 2001. Adequacy of concentrated equine serum product in preventing failure of immune passive transfer in neonatal foals: Preliminary study. *Equine Veterinary Journal*, 33, 734-736.

HAMIR, A.N., VAALA, W., HEYER, G. & MOSER, G., 1994. Disseminated equine herpesvirus-1 infection in a two-year old filly. *Journal of Veterinary Diagnostic Investigation*, 6, 493-496.

HANNANT, D., JESSET, D.M., O'NEILL, T., LIVESAY, G.J. & MUMFORD, J.A., 1994a. Cellular immune responses stimulated by inactivated virus vaccines and infection with equine influenza virus (H3N8). *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994*,* Nakajima, H. & Plowright, W. (eds), 169-174.

HANNANT, D., JESSETT, D.M., O'NEILL, T. & MUMFORD, J.A., 1989. Antibody isotype responses in the serum and respiratory tract to primary and secondary infections with equine influenza virus (H3N8). *Veterinary Microbiology,* 19, 293-303.

HANNANT, D., MUMFORD, J.A. & JESSETT, D.M., 1988. Duration of circulating antibody and immunity following infection with equine influenzavirus. *The Veterinary Record*, 122, 125-128.

HANNANT, D., O'NEILL, T., OSTLUND, E.N., KYDD, J.H. & MUMFORD, J.A., 1994b. Poster abstract: EHV-1 induced immunosuppression is associated with lymphoid cells rather than

circulating antigen/immune complexes. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 355-356.

HANSON, L.K., BINNS, M., MUMFORD, J.A. & APPELTON, J.A., 1998. Molecular basis of apparent antigenic reversion in the equine influenza virus H3 haemagglutinin. *In* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade, J.F., Mumford, J.A. & Kaaden, O-R. (eds), 25-32.

HAYAKAWA, Y., KOMAE, H., IDE, H., NAKAGAWA, H., YOSHIDA, Y., KAMADA, M., KATAOKA, Y. & NAKAZAWA, M., 1993. An occurrence of equine transport pneumonia caused by mixed infection with *Pasteurella caballi. Streptococcus suis* and *Streptococcus zooepidemicus. Journal of Veterinary Medical Science*, 55, 455-456.

HAYFLICK, l., 1965. Tissue cultures and mycoplasmas. *Texas Reports on Biology and Medicine*, 6 Supplement 1, 331-336.

HIGASHI, T. & HARASAWA, R., 1989. DNA restriction analysis of equine adenovirus serotype 1. *Journal of Veterinary Medicine, B,* 36, 473-476.

HOFER, B., STECK, F., GERBER, H., LOHRER, J., NICOLET, J. & PACCAUD, M.F., 1973. An investigation into the aetiology of viral respiratory disease in a remount depot. *In:* Equine infectious diseases III. Proceedings of the 3rd International Conference of Equine Infectious Diseases, Paris, Karger, Basel,.527-545.

HOFFMAN, A.M. , BAIRD, J.D. , KLOEZE, H.J. , ROSENDAL, S. & BELL, M., 1992. *Mycoplasma felis* pleuritis in two show-jumper horses. *Cornell Veterinarian*, 1992, 82, 155-162.

HOFFMAN, A.M. & VIEL, L., 1997. Techniques for sampling the respiratory tract of horses. *Veterinary Clinics of North America: Equine Practice*, 13, 463-475.

HOFFMAN, A.M., VIEL, L., JUNIPER, E. & PRESCOTT, J.F., 1993a. Clinical and endoscopic study to estimate the incidence of distal respiratory tract infection in Thoroughbred foals on Ontario breeding farms. *American Journal of Veterinary Research,* 54, 1602-1607.

HOFFMAN, A.M., VIEL, L. & PRESCOTT, J.F., 1999. Incidence of lower respiratory disorders in large weanling farms: a review. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds),

HOFFMAN, A.M., VIEL, L., PRESCOTT, J.F., ROSENDAL, S. & THORSEN, J., 1993b. Association of microbiologic flora with clinical, endoscopic and pulmonary cytologic findings in foals with distal respiratory tract infection. *American Journal of Veterinary Research*, 54, 1615-1622.

HOFNER, B., STECK, F. & GERBER, H., 1978. Virological investigations in a horse clinic. *In:* Equine Infectious Disease IV. Proceedings of the 4th International Conference, Lyon, 1976, Bryans, J.T. & Gerber, H. (eds). Veterinary Publications, Inc., 475-489.

HOLMES, D.F., KEMEN, M.J. & COGGINS, L., 1978. Equine rhinovirus infection - serologic evidence of infection in selected United States horse populations. *In:* Equine Infectious Diseases IV. Proceedings of the 4th International Conference, Lyon 1976, Bryans, J.T. & Gerber, H. (eds). Veterinary Publications, Inc., 315-319.

HOLT, J.G., & KRIEG, N.R., (eds.), 1984. Bergey's manual of systematic bacteriology, Vol 1. Williams & Wilkins, Baltimore, Hong Kong, London & Sydney.

HOLT, J.G., KRIEG, N.R., SNEATH, P.H.A., STALEY & WILLIAMS, S.T. (eds), 1994. Bergey's Manual of Determinative Bacteriology, 9th Edition. pp. 787. Williams & Wilkins, Baltimore, Hong Kong, London & Sydney.

HOLT, J.G., SNEATH, P.H.A., MAIN, N.S., & SHARPE, M.E. (eds.), 1986, Bergey's Manual of Systematic Bacteriology, Vol 2. Williams & Wilkins, Baltimore, Hong Kong, London, Sydney.

HOLT, J.G., STALEY, J.T., BRYANT, M.P., & PFENNIG, N. (eds.), 1989a. Bergey's Manual of Systematic Bacteriology, Vol 3. Williams & Wilkins, Baltimore, Hong Kong, London, Sydney.

HOLT, J.G., STALEY, J.T., BRYANT, M.P., & PFENNIG, N. (eds.), 1989b. Bergey's Manual of Systematic Bacteriology, Vol 3. Williams & Wilkins, Baltimore, Hong Kong, London, Sydney.

HOLT, J.G., WILLIAMS, S.T., & SHARPE, M.E. (eds.), 1989c. Bergey's Manual of Systematic Bacteriology, Vol 4. Williams & Wilkins, Baltimore, Hong Kong, London & Sydney.

HONDALUS, M. & PARADIS, M.R., 1989. Respiratory disease in foals: the initial steps toward diagnosis. *Veterinary Medicine*, 84, 1168-1173.

HOQUET, F., HIGGINS, R. & LESSARD, R., 1985. Comparison of the bacterial and fungal flora in the pharynx of normal horses and horses affected with a pharyngitis. *Canadian Veterinary Journal,* 26, 342-346.

HOWELL, P.G., GROENEWALD, M., VISAGIE, C.W., BOSMAN, A-M., COETZER, J.A.W. & GUTHRIE, A.J., 2002. The classification of seven serotypes of equine encephalosis and the prevalence of homologous antibody in horses in South Africa. *Onderstepoort Journal of Veterinary Research*. 69, 79–93.

HUGHES, K.L., SULAIMAN, I., 1987. The ecology of *Rhodococcus equi* and physiocochemical influences on growth. *Veterinary Microbiology,* 14, 241-250.

JACOBSZ, E.P., undated. Mycoplasma: Labortorium handleiding. N.I.V., Onderstepoort Veterinary Institute.

JANG, S.S., BIBERSTEIN, E.L. & HIRSH, D.C., 1987. *Actinobacillus suis*-like organisms in horses. *American Journal of Veterinary Research,* 48, 1032–1038.

JEFFCOTT, L.B., 1972. Passive immunity and its transfer with special references to the horse. *Biological Reviews*. 47, 439-464.

JEFFCOTT, L.B., 1974. Some practical aspects of the transfer of passive immunity to newborn foals. *Equine Veterinary Journal*, 6, 109-115.

JOLLY, P.D., ZHEN, F.F. & ROBINSON A.J., 1986. Viruses associated with respiratory disease of horses in New Zealand: an update. *New Zealand Veterinary Journal*, 34, 46-50.

KAMADA, M. & AKIYAMA, Y., 1975. Studies on the distribution of *Streptococcus zooepidemicus* in equine respiratory tracts. *Experimental Reports of Equine Health Laboratory*, 12, 53-63.

KAMADA, M., 1978. Comparison of four serological tests for detecting antibodies against equine adenovirus. *Experimental Reports of Equine Health Laboratory* 15, 91-99.

KAMADA, M. & AKIYAMA, Y., 1975. Studies on the distribution of *Streptococcus zooepidemicus* in equine respiratory tracts. *Experimental Reports of the Equine Health Laboratory*, 12, 53-63.

KAMADA, M., AKIYAMA, Y. & SATO, K., 1977. Isolation of adenovirus from adult Thoroughbred horses. *Japanese Journal of Veterinary Science* 39, 661-662.

KERSHAW, O., VON OPPEN, T., GLITZ, F., DEEGEN, E., LUDWIG, H. & BORCHERS, K., 2001. Detection of equine herpesvirus type 2 (EHV-2) in horses with keratoconjunctivitis. *Virus Research*, 80, 93-99.

KONEMAN, E.W., ALLEN, S.D., JANDA, W.M., SCHREDENBERGER, P.C. & WINN, J.W.C. (eds.), 1995. Colour Atlas and Textbook of Diagnostic Microbiology, 4th Edition. J B Lippincott Company, Philadelphia.

KORENEK, N.L., LEGENDRE, A.M., ANDREWS, F.M., BLACKFORD, J.T., WAN, P.Y., BREIDER, M.A. & RINALDI, M.G., 1994. Treatment of mycotic rhinitis with itraconazole in three horses. *Journal of Veterinary Internal Medicine*, 8, 224-227.

KOTERBA, A.M., 1990a. Respiratory disease: Approach to diagnosis. *In:* Equine Clinical Neonatology**.** Koterba, A.M., Drummond, W.H. & Kosch, P.C. (eds), Lea & Febiger, Philadelphia, London, 153-176.

KOTERBA, A.M., 1990b. Diagnosis and management of the normal and abnormal neonatal foal: general considerations*. In***:** Equine Clinical Neonatology*.* Koterba, A.M., Drummond, W.H. & Kosch, P.C. (eds), Lea & Febiger, Philadelphia, London, 3-15.

KYDD, J.H., SMITH, K.C., HANNANT, D., LIVESAY, G.J. & MUMFORD, J.A., 1994. Distribution of equid herpesvirus-1 (EHV-1) in the respiratory tract of ponies: implications for cellular immunity. *Equine Veterinary Journal*, 26, 470-473.

LARONE, D.H., 1995. Medically Important Fungi: A Guide to Identification, 3rd Edition. American Society of Microbiology Press, Washington D.C..

LAVOIE, J.P., COUTURE, L., HIGGINS, R. & LAVERTY, S., 1991. Aerobic bacterial isolates in horses in a university hospital, 1986-1988. *Canadian Veterinary Journal*, 32, 292-294.

LAWRENCE, G.L., GILKERSON, J., LOVE, D.N., SABINE, M. & WHALLEY, J.M., 1994. Rapid, single-step differentiation of equid herpesvirus 1 and 4 from clinical material using the polymerase chain reaction and virus specific primers. *Journal of Virological Methods* 47, 59-72.

LEADON, D.P., 1989. Bacteriology and pathogenesis of *Rhodococcus equi*. *Equine Veterinary Education*, 1, 25-26.

LE BLANC, M.M., TRAN, T., BALDWIN, J.L. & PRITCHARD, E.L., 1992. Factors that influence passive transfer of immunoglobulins in foals. *Journal of the American Veterinary Medical Association*, 200, 179-183.

LÉGUILLETTE, R., ROY, M.F. & LAVOIE, J-P., 2002. Foal pneumonia. *In*: Equine Respiratory Diseases, LEKEUX, P. (ed). *International Veterinary Information Service* (www.ivis.org), Ithaca, New York, USA.

LENNETTE, E.H., 1969. Chapter one. General principals underlying laboratory diagnosis of viral and rickettsial infections. *In*: Diagnostic Procedures for Viral and Rickettsial Infections, 4th Edition, Lennette, E., H. & Schmidt, N.J., (eds), American Public Health Association, Inc., 1-65.

LENNETTE, E.H., BALOWS, A., HAUSLER, W.J. & SHADONY, H.J. (eds), 1985. Manual of Clinical Microbiology, 4th edition, American Society for Microbiology, Washington, DC..

LENNETTE, E.H., HALONEN, P. & MURPHY, F.A. (eds), 1988. Laboratory diagnosis of infectious diseases: principals and practice. Volume II: viral, rickettsial and chlamydial diseases. Springer-Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo.

LI, F., DRUMMER, H.E., FICORILLI, N., STUDDERT, M.J. & CRABB, B.S., 1997. Identification of non-cytopathogenic equine rhinovirus-1 as a cause of acute febrile respiratory disease in horses. *Journal of Clinical Microbiology*, 35, 937-943.

LINDSTROM, S., ENDO, A., SUGITA, S., PECORARO, M., HIROMOTO, Y., KAMADA, M., TAKAHASHI, T. & NEROME, K., 1998. Phylogenetic analyses of the matrix and non-structural genes of equine influenza viruses. *Archives of Virology*, 143, 1585-1598.

LIVESAY, J., O'NEILL, T., HANNANT, D., YADAV, M.P. & MUMFORD, J.A., 1993. The outbreak of equine influenza (H3N8) in the United Kingdom in 1998; diagnostic use of an antigen capture ELISA. *The Veterinary Record*, 133, 515-519.

MACHANG'U, R.S. & PRESCOTT, J.F., 1991. Purification and properties of cholesterol oxidase and choline phosphohydrolase from *Rhodococcus equi*. *Canadian Journal of Veterinary Research*. 55, 332-340.

MANNHEIM, W., 1984. Family III. Pasteurellaceae. *In*: Bergey's Manual for Systematic Bacteriology, Vol 1, Holt, J. & Krieg, N. R. (eds). The Williams & Wilkins Co., Baltimore, Hong Kong, London & Sydney, 550-558.

MARTENS, J.G. & MARTENS, R.J., 1991. Equine herpesvirus type 1: Its classifications, pathogenesis, and prevention. *Veterinary Medicine*, September, 936-940.

MARTENS, R.J., RUOFF, W.W. & RENSHAW H.W., 1981. Foal pneumonia: a practical approach to diagnosis and therapy. *Compendium for Continuing Education*, 4, 217-229.

MASON, D.K., WATKINS, K.L., McNIE, J.T. & LUK, C.M., 1990. Haematological measurements as an aid to early diagnosis of respiratory viral infections in Thoroughbred horses. *The Veterinary Record,* 126, 359-363.

MATSUMURA, T., KIRISAWA, R., KONDO, T., SUGITA, S. & KAMADA, M., 1994. Molecular epizootiological studies on respiratory and foetal strains of equid herpesvirus-1 isolated in Japan. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 243-249.

MATSUMURA, T., SUGIURA, T., IMAGAWA, H.L., FUKUNAGA, Y. & KAMADA, M., 1992. Epizootiological aspects of type 1 and type 4 equine herpesvirus infections among horse populations. *Journal of Veterinary Medical Science*, 54, 207-211.

McCHESNEY, A.E., 1975. Viral respiratory infections of horses: structure and function of lungs in relation to viral infection. *Journal of the American Veterinary Medical Association*, 166, 76-77.

McCOLLUM, W.H., 1992. Equine rhinovirus-1. Abstract: *Equine Veterinary Data* 13, 53.

McCOLLUM, W.H. & BRYANS, J.T., 1972. Serological identification of infection by equine arteritis virus in horses of several countries. *In:* Equine Infectious Diseases III. Proceedings of the 3rd International Conference, Paris, Karger, Basel. 256-260.

McCOLLUM, W.H., GILBERT, S.A., DEREGT, D. & TIMONEY, P.J., 1999. Detection of equine arteritis virus in blood and respiratory tract secretions of experimentally infected horses by a nested PCR assay. *In*: Equine Infectious Diseases VIII. Proceedings of the 8th International Conference,

Dubai, 23rd-26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 434-435.

McCOLLUM, W.H., PRICKETT, M.E. & BRYANS, J.T., 1971. Temporal distribution of EVA in respiratory mucosa, tissues and body fluids of horses infected by inhalation. *Research in Veterinary Science*, 2, 459-464.

McCOLLUM, W. H., TIMONEY, P. J., ROBERTS, A. W., WILLARD, J. E. & CARSWELL, G. D., 1988. Response of vaccinated and non-vaccinated mares to artificial insemination with semen from stallions persistently infected with equine arteritis virus. *In*: Equine infectious diseases V. Proceedings of the 5th International Conference, Lexington. Powell D.G. (ed). University Press of Kentucky, 13-18.

McGUIRE, T.C. & CRAWFORD, T.B., 1973. Passive immunity in the foal: measurement of immunoglobulin classes and specific antibody. *American Journal of Veterinary Research*, 34, 1299-1303.

MEISWINKEL, R., NEVILL, E.M. & VENTER, G.J., 1994. Vectors: *Culicoides* spp. *In:* Infectious Diseases of Livestock with Special Reference to Southern Africa. Volume one. Coetzer, J.A.W., Thomson G.R. & Tustin, R. C. (eds), Oxford University Press, Cape Town, 68-89.

MEISWINKEL, R. & PAWSEKA, J.T., 2003. Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. *Preventive Veterinary Medicine*, 60, 243-253.

MEYER, H., THEIN, P. & HÜBERT, P., 1987. Characterization of two equine herpesvirus (EHV) isolates associated with neurologic disorders in horses. *Journal of Veterinary Medicine B*, 34, 545.

MEYER, J.C., KOTERBA, A., LESTER, G. & PURICH, B.L., 1992. Bacteraemia and pneumonia in a neonatal foal caused by *Streptococcus pneumonia* type 3. *Equine Veterinary Journal*. 24, 407-410.

MORRIS ANIMAL FOUNDATION, 1978. Report of the foal pneumonia panel. *Journal of Equine Medicine*, 2, 400-404, 412, 428-433.

MUMFORD, J.A., 1994a. Equine Rhinovirus Infections. *In*: Infectious Diseases of Livestock: with Special Reference to Southern Africa, Volume two. Coetzer, J.A., Thomson, G.R., Tustin, R.C. & Kriek, N.P.J. (eds.). Oxford University Press. Cape Town. 820-822.

MUMFORD, J.A., 1994b. Equine influenza. *In*: Infectious Diseases of Livestock with Special Reference to Southern Africa, Volume two. Coetzer, J.A., Thomson, G.R. & Tustin, R.C. (eds.). Oxford University Press. Cape Town. 854-859.

MUMFORD, J.A., 1999. Control of influenza from an international perspective. *In*: Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 11-24.

MUMFORD, J.A. & BATES, J. 1984. Trials of an inactivated equid herpesvirus 1 vaccine: Challenge with subtype 2 virus. *The Veterinary Record*, 114, 375-381.

MUMFORD, J.A. & ROSSDALE, P.D., 1980. Virus and its relationship to the "poor performance" syndrome. *Equine Veterinary Journal*, 12, 3-9.

MUMFORD, J.A. & THOMSON, G.R., 1978a. Serological methods for the identification of slowly growing herpesvirus isolated from the respiratory tract of horses. *In*: Equine Infectious Diseases IV. Proceedings of the 4th International Conference, Lyon, 1976, Bryans, J. T. & Gerber, H. (eds), Veterinary Publications, Inc., 49-52.

MUMFORD, J. A., & THOMSON, G. R., 1978b. Studies on picornavirus isolated from the respiratory tract of horses. *In:* Equine Infectious Diseases IV. Proceedings of the 4th International Conference on Equine Infectious Disease, Lyon, 1976. Bryans, J. T. & Gerber, H. (eds), Veterinary Publications, Inc., 419-429.

MUMFORD, E.L., TRAUB-DARGATZ, J.L., SALMAN, M.D., COLLINS, J.K., GETZEY, D.M. & CARMAN, J., 1998. Monitoring and detection of acute viral respiratory tract disease in horses. *Journal of the American Veterinary Medical Association,* 213, 385-390.

MUMFORD, J.A., WOOD, J.M., SCOTT, A.M., FOLKERS, C. & SCHILD, G.C., 1983. Studies with inactivated equine influenza vaccine. 2. Protection against experimental infection with influenza virus A/equine/Newmarket/79 (H3N8). J*ournal of Hygiene, Cambridge*, 90, 385-395.

MURRAY, M.J., 1989. Respiratory problems in horses: dealing with lower airway disease. *Veterinary Medicine*, 105-110.

NEU, S.M., TIMONEY, P.J. & McCOLLUM, W.H., 1988. Persistent infection of the reproductive tract in stallions persistently infected with equine arteritis virus. *In:* Equine Infectious Diseases V. Proceedings of the 5th International Conference, Lexington, 1987, Powell, D.G. (ed). University Press of Kentucky, 3-12.

NEWTON, J.R., WOOD, J.L.N., DEBRAUWERE, M.N., CHANTER, N., VERHEYEN, K. & MUMFORD, J.A., 1998. Detection and treatment of asymptomatic carriers of *Streptococcus equi* following strangles outbreaks in the UK. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998. Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 82-87.

NORDENGRAHN, A., RUSVAI, M. & MERZA, M., 1996. Equine herpesvirus type 2 (EHV-2) as a predisposing factor for *Rhodococcus equi* pneumonia in foals: prevention of the bifactorial disease with EHV-2 immunostimulating complexes. *Veterinary Microbiology*, 51, 55-68.

OGILVIE, T.H., ROSENDAL, S., BLACKWELL, T.E., ROSTKOWSKI, C.M., JULIAN, R.J. & RUHNKE, L., 1983. *Mycolasmas felis* as a cause of pleuritis in horses. *Journal of the American Veterinary Medical Association*, 182, 1374-1376.

OSTLUND, E.N., 1993. The equine herpesviruses. *Veterinary Clinics of North America: Equine Practice*, 9, 283-294.

OXBURGH, L. & HAGSTROM, A., 1999. A PCR based method for the identification of equine influenza virus from clinical samples. *Veterinary Microbiology*, 67, 161-174

PACE, L.W., WILSON, D.A., FALES, W.H., MESSER, T., LATTIMER, J.C. & MATTHEWS, G.L., 1995. *Salmonella* septicaemia with pulmonary abscesses and osteomyelitis in a foal. *Equine Veterinary Education*, 7, 64-66.

PÄLFI V., BELÄK, S. & MOLNÁR, T., 1978. Isolation of equine herpesvirus type 2 from foals, showing respiratory symptoms. *Zentralblatt für Veterinärmedizin B,* 25, 165-167*.*

PAVRI, K.M. & APTE, V.H., 1967. Isolation of *Pasteurella multocida* from a fatal disease of horses and donkeys in India. *The Veterinary Record*, 80, 437-439.

PHILLIPS, J.E., 1984. Genus III. *Actinobacillus*. In*:* Bergey's Manual of Systematic Bacteriology, Vol 1, Baltimore. Holt, J. & Krieg N.R. (eds), The Williams & Wilkins Co., Baltimore, Hong Kong, London & Sydney, 570-575.

PLATEAU, E. & CRUCIERE, C., 1983. Study on radial haemolysis method for the detection of anti influenza equi antibodies in equine sera: reliability and expression of results. *Zentralblatt für Veterinärmedizin B*, 30, 512-520.

PLATT, H., 1973. Septicaemia in the foal. A review of 61 cases. *British Veterinary Journal*, 129, 221-229.

PLATT, H., 1977. Joint-ill and other bacterial infections on Thoroughbred studs. *Equine Veterinary Journal*, 9, 141-145.

PLUMMER, G. & KERRY, J.B., 1962. Studies on an equine respiratory virus. *The Veterinary Record,* 74, 967-970.

POWELL, D.G., 1994. Panel Discussion summary: International movement and equine infectious diseases. *In:* Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 288.

POWELL, D.G., 1991. Viral Respiratory Disease in the Horse. *Veterinary Clinics of North America: Equine Practice*, 17, 27-50.

POWELL, D.G., BURROWS, R. & GOODRIDGE, D., 1974. Respiratory viral infections among Thoroughbred horses in training during 1972. *Equine Veterinary Journal*, 6, 19-24.

POWELL, D.G. , BURROWS, R., SPOONER, P.R., GOODRIDGE, D., THOMSON, G.R. & MUMFORD, J., 1978. A study of infectious respiratory disease among horses in Great Britain, 1971-1976. *In*: Equine infectious diseases IV. Proceedings of the 4th International Conference on Equine Infectious Diseases, Lyon, 1976. Bryans, J.T. & Gerber, H. (eds), Veterinary Publications, Inc., 451-459.

QUINN, P.J., CARTER, M.E., MARKEY, B. & CARTER, G.R. (eds.), 1994. Clinical Veterinary Microbiology. Wolfe Publishing, London, United Kingdom.

RANDOLPH, J.F., MOISE, N.S., SCARLETT, J. M., SHIN, S.J., BLUE, J.T. & BOOKBINDER P.R., 1993. Prevalence of mycoplasmal and ureaplasmal recovery from tracheobronchial lavages and prevalence of mycoplasmal recovery from pharyngeal swab specimens in dogs with or without pulmonary disease. *American Journal of Veterinary Research*, 54, 387-391.

REZABEK, G.B., DONAHUE, J.M., GILES, R.C., PETRITES-MURPHY, M.B., POONACHA, K.B., ROONEY, J.R., SMITH, B.J., SWERCZEK, T.W. & TRAMONTIN, R.R., 1993. Histoplasmosis in horses. *Journal of Comparative Pathology*, 109, 47-55.

RILEY, C.B., BOLTON, J.R., MILLS, J.N. & THOMAS, J.B., 1992. Cryptococcosis in seven horses. *Australian Veterinary Journal*, 69, 135-139.

RIPPON, J.W., 1982. Medical Mycology: The Pathogenic Fungi and Pathogenic Actinomycetes, 2nd Edition, W. B. Saunders Co., Philadelphia, London, Toronto, Mexico City, Rio de Janeiro, Sydney, Tokyo.

ROBINSON, J.A., ALLEN, G.K., GREEN, E.M., FALES, W.H., LOCH, W.E. & WILKERSON, C.G., 1993. A prospective study of septicaemia in colostrum-deprived foals. *Equine Veterinary Journal*, 25, 214-219.

ROGERS, A. L., 1988. A-equi-2 influenza in horses in the Republic of South Africa. *Journal of the South African Veterinary Association*, 59, 123-125.

ROIZMAN, B., 1996. Herpesviridae: *In:* Virology, 3rd Edition. Field, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melnick, J.L., Monath, T.P., Roizman, B. & Straus, S.E. (eds), Lippencott-Raven, Philadephia, New York, 2221-2230.

ROSENDAL, S., BLACKWELL, T.E., LUMSDEN, J.H., PHYSICK-SHEARD, P.W., VIEL, L., WATSON, S. & WOODS, P., 1986. Detection of antibodies to *Mycoplasma felis* in horses. *Journal of the American Veterinary Medical Association*, 188, 292-294.

ROUSE, B.T. & DITCHFIELD, W.J.B., 1970. Response of ponies to *Myxovirus influenzae* A-Equi-2: The protective effect of serum and nasal antibody against experimental challenge. *Research in Veterinary Science,* 11, 503-507.

RUMBAUGH, G.E., ARDANS, A.A., GINNO, D. & TROMMERSHAUSEN, S.A., 1979. Identification and treatment of colostrum-deficient foals. *Journal of the American Veterinary Medical Association*, 174, 273-276.

RYAN, J.A., MODRANSKY, P.D. & WELKER, B., 1992. Guttural pouch mycosis in a 3-month-old foal. *Equine Practice*, 14, 21-22.

SAMITZ, E.M. & BIBERSTEIN, E.L., 1991. *Actinobacillus suis*-like organisms and evidence of haemolytic strains of *Actinobacillus lignieresii* in horses. *American Journal of Veterinary Research*, 52, 1245-1251.

SARASOLA, P., TAYLOR, D.J., LOVE, S. & McKELLAR, Q.A., 1992. Short communications: Secondary bacterial infections following an outbreak of equine influenza. *The Veterinary Record*, 131, 441-442.

SAXEGAARD, F. & SVENKERUD, R., 1974. *Pasteurella haemolytica* associated with pneumonia in a foal: a case report. *Acta Veterinaria Scandinavica*, 15, 439-441.

SCHILD, G.C., PEREIRA, M.S. & CHAKRAVERTY, P., 1975. Single radial-haemolysis: a new method for the assay of antibody to influenza haemaggltinin. *Bulletin of the World Health Organisation*, 52, 43-50.

SCHLATER, L.R.K., 1989. An aerogenic *Pasteurella*-like organism isolated from horses. *Journal of Veterinary Investigation*, 1, 3-5.

SCHMIDT, N.J., 1976. Antibody assays for enteroviruses and reoviruses. *In*: Manual of Clinical Immunology, Rosen, N.R. & Friedman, H. (eds), American Society of Microbiology, Washington DC, 488-493.

SCOTT, J.C., DUTTA, S.K. & MYRUP, A.C., 1983. *In vivo* harbouring of equine herpesvirus-1 in leukocyte populations and subpopulations and their quantification from experimentally infected ponies. *American Journal of Veterinary Research*, 44, 1344-1348.

SENNE, D.A., PEARSON, J.E. & CARBEY, E.A., 1985. Equine viral arteritis: A standard procedure for the virus neutralisation and comparison of results of a proficiency test performed at five laboratories. *Proceedings of the United States Animal Health Association*, 29-34.

SHERMAN, J., MITCHELL., W.R., MARTIN, S.W., THORSEN, J., & INGRAM, D.G., 1979. Epidemiology of equine upper respiratory disease on Standardbred racetracks. *Canadian Journal of Companion Animal Medicine*, 43, 1-9.

SHEORAN, A.S., TIMONEY, J.F., HOLMES, M.A., KARZENSKI, S.S. & CRISMAN, M.V., 2000. Immunoglobulin isotypes in sera and their neonatal transfer and distribution in horses. *American Journal of Veterinary Research*, 61, 1099-1105.

SHIMIZU, A., KAWANO, J., OZAKI, J., SASAKI, N., KIMURA, S., KAMADA, M., ANZAI, S., SAITO, H. & SATO, H., 1991. Characteristics of *Staphylococcus aureus* isolated from lesions of horses. *Journal of Veterinary Medical Science*, 53, 601-606.

SHIMOZAWA, K., ANZAI, T., KAMADA, M. & TAKATORI, K., 1997. Fungal and bacterial isolation from racehorses with infectious dermatosis. *Journal of Equine Science*, 8, 89–93.

SINGH, B.K., 1997. Serological observations of an epidemic of equine influenza in India. *Journal of Equine Veterinary Science*, 17, 380-382.

SLATER, J.D., BAXI, M., TEWARI, D., GIBSON, J.S., FIELD, H.J., LUDWIG, H., STEINBACH, F. & BORCHERS, K., 1994a. Experimental infection of specific pathogen-free ponies with equid herpesvirus-1: Detection of infectious virus and viral DNA. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 255-260.

SLATER, J.D., BORCHERS, K., THACKRAY, A.M. & FIELD, H.J., 1994b. The trigeminal ganglion is a location for equine herpesvirus 1 (EHV-1) latency and reactivation in the horse. *Journal of General Virology*. 75, 2007-2016.

SMITH, B. P. & ROBINSON, R. C., 1981. Studies on an outbreak of *Corynebacterium equi* pneumonia in foals. *Equine Veterinary Journal*, 13, 223-228.

STECK, F., HOFER, B., SCHAREREN, B., NICOLET, J. & GERBER, H., 1978. Equine rhinoviruses: new serotypes*. In*: Equine Infectious Diseases IV. Proceedings of the 4th International Congress, Lyon, 1976, Bryans, J.T. & Gerber, H. (eds), Veterinary Publications, Inc., 321-328.

STERNBERG, S., 1998. isolation of *Actinobacillus equuli* from the oral cavity of healthy horses and comparison of isolates by restriction enzyme digestion and pulsed-field gel electrophoresis. *Veterinary Microbiology*, 59, 147-156.

STOKES, A., CORTEYN, A.H. & MURRAY, P.K., 1991. Clinical signs and humoral response in horses following equine herpesvirus type-1 infection and their susceptibility to equine herpesvirus type-4 challenge. *Research in Veterinary Science*, 51, 141-148.

STUDDERT, M.J., 1971. Equine herpesviruses 4. Concurrent infection in horses with strangles and conjunctivitis. *Australian Veterinary Journal*, 47, 434-436.

STUDDERT, M.J., 1996. Editorials: Equine herpesvirus 2 and disease. *Equine Veterinary Journal*, 28, 426-428.

STUDDERT, M.J., 2003. Equine adenoviruses. *In*: Equine Respiratory Diseases, Lekeux, P. (ed). International Veterinary Information Service, Ithaca New York. www.ivis.org.

STUDDERT, M.J., HARTLEY, C.A., DYNON, K., SANDY, J.R., SLOCOMBE, R.F., CHARLES, J.A., MILNE, M.E., CLARKE, A.F. & EL-HAGE, C., 2003, Outbreak of equine herpesvirus type 1 myeloencephalitis: new insights from virus identification by PCR and the application of an EHV-1-specific antibody detection ELISA. *The Veterinary Record*, 153, 417-423.

STUDDERT, M.J., TURNER, A.J. & PETERSON, J.E., 1970. Equine herpesviruses, 1. Isolation and characterisation of equine rhinopneumonitis virus and other equine herpesviruses from horses. *Australian Veterinary Journal*, 46, 83-89.

STUDDERT, M.J. & GLEESON, L.J., 1978. Isolation and characterisation of an equine rhinovirus. *Zentralblatt für Veterinärmedizin B*, 25, 225-237.

STUDDERT, M.J., WILKS, C.R. & COGGINS, L., 1974. Antigenic relationships and serologic survey of equine adenoviruses. *American Journal of Veterinary Research,* 35, 693-670.

STYLIANIDES, E., **2000**. Standard operating procedures for the complement-fixation test. Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. 1-7.

SUGIURA, T., FUKUZAWA, Y., KAMADA, M., ANDO, Y. & HIRASAWA, K., 1983. Isolation of Equine Herpesvirus Type 2 from foals with pneumonitis. *Bulletin of the Equine Research Institute*, 20, 148-153.

SUGIURA, T., MATSUMURA, T., FUKUANYA, Y. & HIRASAWA, K., 1987. Sero-epizootioloigcal study of racehorses with pyrexia in the training centres of the Japan racing association**.** *Japanese Journal of Veterinary Science*, 49, 1087-1096.

SUGIURA, T., MATSUMURA, T. & IMAGAWA, H., 1988a. A 7-year serological study of viral agents causing respiratory infection with pyrexia among racehorses in Japan. *In*: Infectious Diseases V. Proceedings of the 5th International Conference Equine Infectious Diseases, Lexington, Powell, D.G., (ed). University Press of Kentucky, 258-261.

SUGIURA, T., MATSUMURA, T. & HIRANO, S., 1988b. Field surveillance of equine herpesvirus type 1 infection in racehorses by agar gel immunodiffusion test. *Bulletin of Equine Research*, 25, 15-9.

SUTTON, G.A., VIEL, P.S., CARMAN, P.S. & BOAG, B.L., 1998. Pathogenesis and clinical signs of equine herpesvirus-1 in experimentally infected ponies *in vivo*. *Canadian Journal of Veterinary Research*, 62, 49-55.

SWEENEY, C.R., DIVERS, T.J. & BENSON, C.E., 1985. Anaerobic bacteria in 21 horses with pleuropneumonia. *Journal of the American Veterinary Medical Association*, 7, 712-724.

SWEENEY, C.R. & HABECKER, P.L., 1999. Pulmonary aspergillosis in horses: 29 cases (1974-1997). *Journal of the American Veterinary Medical Association*, 214, 808-811.

SWEENEY, C.R., HOLCOMBE, S.J., BARNINGHAM, S.C. & BEECH, J., **1991**. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the isolates. *Journal of the American Veterinary Medical Association*, 198, 839-842.

SWEENEY, C.R. SWEENEY, R.W. & BENSEN, C.E., 1989. Comparison of bacteria isolated from specimens obtained by the use of endoscopic guarded tracheal swabbing and percutaneous tracheal aspiration in horses. *Journal of the American Veterinary Medical Association,* 195*,* 1225-1229.

TAKAI, S., FUJIMORI, T., KATSUZAKI, K. & TSUBAKI, S., 1987. Ecology of *Rhododocccus equi* in horses and their environment on horse breeding farms. *Veterinary Microbiology*, 14, 233-239.

TAKAI, S., TAKAHAGI, J., SATO, Y., YAMAGUCHI, K., KAKIZAKI, S., TAKEHARA, F., MATSUKURA, S., TAMADA. Y., TANI, A., SASAKI, Y., TSUBAKI, S. & KAMADA, M., 1994. Molecular epidemiology of virulent *Rhodococcus equi* in horses and their environment. *In*: Equine Infectious Diseases VII. Proceedings of the 7th International Conference, Tokyo, 8th – 11th June 1994, Nakajima, H. & Plowright, W. (eds), 183-187.

TANIGUCHI, A., FUKUSHI, HG., YANAI, T., MASEGI, T., YAMAGUCHI, T. & HIRAI, K., 2000. equine herpesvirus 9 induced lethal encephalomyelitis in experimentally infected goats. *Archives of Virology*, 145, 2619-2627.

TANNER, A.C., 1982. *Microsporum gypseum* as the cause of ringworm in a horse. *The Veterinary Record*, 111, 396.

TEARLE, J.P., SMITH, K.C., BOYLE, M.S., BINNS, M.M., LIVESAY, G.J. & MUMFORD, J.A., 1996. Replication of equid herpesvirus-1 (EHV-1) in the testes and epididymides of ponies and venereal shedding of infectious virus. *Journal of Comparative Pathology*, 115, 385-397.

TELFORD, E.A.R., STUDDERT, M.J., AGIUS, C.T., WATSON, M.S., AIRD, H.C. & DAVIDSON, A.J., 1993. Equine herpesviruses 2 and 5 are γ-herpesviruses. *Virology*. 195, 492-499.

THEIN, P. & BÖHM, D., 1976. Ätiologie und Klinik Einer Virusbedingten Keratokonjunktivitis beim Fohlen. *Zentralblatt für Veterinärmedizin B*, 23, 507-519.

THOMSON, G.R., 1978. The role of equid herpesvirus 1 in acute respiratory disease of horses and the use of inactivated antigens to immunize against infection. PhD thesis, University of London.

THOMSON, G.R., MUMFORD, J.A., CAMPBELL, J., GRIFFITHS, L. & CLAPHAM, P., 1976. Serological detection of equid herpesvirus-1 infections of the respiratory tract*. Equine Veterinary Journal*, 8, 58-65.

THORSEN, J., 1991. Equine rhinoviruses. *Equine Practice*, 13, 19-22.

TIMONEY, J.F., 1993. *Streptococcus*. *In*: Pathogenesis of Bacterial Infections in Animals, 2nd Edition. Iowa State University Press. Gyles, C.J. & Thoen, C.O. (eds), 3-20.

TIMONEY, J.F., 1994. The role of *Streptococcus equi* subsp. *zooepidemicus* in lower respiratory tract infections in foals. *In*: Equine infectious diseases VII. Proceedings of the 7th International Conference, Tokyo, Japan 8th - 11th June 1994, Nakajima, H. & Plowright, W. (eds), 319.

TIMONEY, J.F., ARTIUSHIN, S. & WANG, J., 1998. Regions of the protective M-like protein of *Streptococcus equi* recognised by serum and mucosal antibodies of convalescent horses. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 88-94.

TIMONEY, J.F., UMBACH, A., BOSCHWITZ, J.E. & WALKER, J.A., 1994. *Streptococcus equi* subsp. *equi* expresses 2 M-like proteins including a homologue of the variable M-like protective protein of subsp. *zooepidemicus*. *In:* Equine infectious diseases VII. Proceedings of the 7th International Conference, Tokyo, Japan 8th - 11th June 1994, Nakajima, H. & Plowright, W. (eds), 189-193.

TIMONEY, P.J., 1996. Equine influenza. *Comparative Immunology and Microbiology of Infectious Diseases,* 19, 205-211.

TIMONEY, P.J., McCOLLUM, W.H., ROBERTS, A.W. & MURPHY, T.W., 1986. Demonstration of the carrier state in naturally acquired equine arteritis infection in the stallion. *Research in Veterinary Science*, 41, 279-280.

TODD, J.D., 1969. Comments on rhinoviruses and parainfluenza viruses of horses. *Journal of the American Veterinary Medical Association*, 155, 387-390.

TRAUB-DARGATZ, J., 1997. Field examination of the equine patient with nasal discharge. *Veterinary Clinics of North America: Equine Practice*, 13, 561 – 588.

TÚMOVÁ, B., 1980. Equine influenza: A segment in influenza virus ecology. *Comparative Immunology, Microbiology, Infection and Disease*, 3, 45-49.

TURNER, A.J., STUDDERT, M.J. & PETERSEN, J.E., 1970. Persistence of equine herpesviruses in experimentally infected horses and experimental induction of abortion. *Australian Veterinary Journal*, 46, 90-98.

VARMA, K.J., POWERS, T.E., POWERS, D.J. & SPURLOCK, S.L., 1984. Standardization of an experimental disease model of *Streptococcus zooepidemicus* in the equine. *Journal of Veterinary Pharmacology and Therapeutics*, 7, 183-188.

VENTER, B.J., GROENEWALD, D.M., PAWESKA, J.T., VENTER, E.H. & HOWELL, P.G., 1999. Vector competence of selected South African *Culicoides* species for the Bryanston serotype of equine encephalosis virus. *Medical and Veterinary Entomology,* 13, 393-400.

WADELL, G.H., TEIGLAND, M.B. & SIEGEL, M.M., 1963. A new influenza virus associated with equine respiratory disease. *Journal of the American Veterinary Medical Association*, 143, 587-590.

WEBSTER, R.G. & GUO, Y.J., 1991. New influenza in horses. *Nature*, 351, 527.

WELCH, H.M., BRIDGES, C.G., LYON, A.M., GRIFFITHS, L. & EDINGTON, N., 1992. Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co-cultivation form lymphoid tissues. *Journal of General Virology*, 73, 261-268.

WELSH, R.D., 1984. The sgnificance of *Streptococcus zooepidemicus* in the horse. *Equine Practice*, 6, 6-16.

WERNEY, U., WERNEY, R., ZACHARIAH, R. & HAYN-EVANS, J., 1998. Serological survey of some equine infectious diseases in the United Arab Emirates. *In:* Equine Infectious Diseases VIII. Proceedings of the 8th International Conference, Dubai, 23rd - 26th March 1998, Wernery, U., Wade J.F., Mumford, J.A. & Kaaden, O-R. (eds), 367-370.

WILKS, C.R. & STUDDERT, M.J., 1972. Isolation of Equine adenovirus*. Australian Veterinary Journa*l, 48, 580-581.

WILLOUGHBY, R., ECKER, G., MCKEE, S., RIDDOLLS, L., VERNAILLEN, C., DUBVI, E., LEIN, D., MAHONY, J.B., CHERNESKY, M., NAGY, W. & STAEMPFI, H., 1992. The effects of equine rhinovirus, influenza virus and herpesvirus infection on tracheal clearance rate in horses. *Canadian Journal of Veterinary Research,* 56, 115-121.

WILSON, J.C., BRYANS, J.T., DOLL, E.R. & TUDOR, L., 1964. Isolation of a newly identified equine respiratory virus. *Cornell Veterinarian,* 55, 425-431.

WILSON, W.D., 1993. Equine influenza. *Veterinary Clinics of North America*: *Equine Practice*, 9, 257-282.

WOOD, J.J.N., BURRELL, M.H., ROBERTS, C.A., CHANTER, N. & SHAW, Y., 1993. Streptococci and *Pasteurella* spp. associated with disease of the equine lower respiratory tract, *Equine Veterinary Journal*, 25, 314-318.

WOOD, J.L.N., CHANTER, N., NEWTON, J.R., BURRELL, M.H., DUGDALE, D., WINDSOR, H.M., WINDSOR, G.D., ROSENDAL, S. & TOWNSEND, H.G.G., 1997. An outbreak of respiratory disease in horses associated with *Mycoplasmas felis* infection. *The Veterinary Record*, 140**,** 388-391.

WOOLCOCK, J.B., 1975. The epidemiology of equine streptococci. *Research in Veterinary Science,* 18, 113-114.

YEARGAN, M.R., ALLEN, G.P. & BRYANS, J.T., 1985. Rapid serotyping of equine herpesvirus-1 isolates with monoclonal antibodies. *Journal of Clinical Microbiology*, 21, 694-697.

YUROV, K.P., ZAGEBINA, E.F. & YARNIKH, E.V., 1997. Equine influenza in Russia and efficacy of vaccination. *In:* Proceedings of the 5th Congress of the World Veterinary Association, Padova, Italy, September 10-12, 1997.

ZSCHOKKE, E., 1913. Em Rhinosporidium beim Pferde. *Schweizer Archiv für Tierheilkunde,* 55, 641-650.