Equine Adenoviruses (15-Apr-2003)

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Summary
Equine adenovirus 1 (EAdV1) occurs worldwide and causes acute upper respiratory tract disease, follicular conjunctivitis, bronchopneumonia and infection of the gastrointestinal tract that is associated with production of soft feces. EAdV1 is peculiarly associated as a dominant pathogen in the uniformly fatal, inherited disease, termed primary severe combined immunodeficiency disease (PSCID) that affects certain Arabian foals. In these foals there is an inexorably progressive EAdV1 bronchopneumonia as well as EAdV1 caused pathology in many other organs and tissues. A second serotype, EAdV2 has been isolated from the feces of foals with diarrhea. Because adenoviruses cause significant respiratory and enteric disease in conventional foals and are the major contributing factor to the death of PSCID foals, comment is made on the view that genetically engineered adenoviruses are suitable as vectors for delivery of foreign genes in the context of vaccines or the correction of genetic defects.

List of Abbreviations
DNA-PKcs - DNA dependent protein kinase catalytic subunit
EAdV1 - Equine Adenovirus 1
EAdV2 - Equine Adenovirus 2
EFK - Equine Fetal Kidney
HI - Hemagglutination Inhibition
K - Kilodaltons
LB - Lymphocyte Blastogenesis
PSCID - Primary Severe Combined Immunodeficiency disease
RAG - Recombination-activating Gene
SN - Serum Neutralization
TCR - T Cell Receptor

Introduction
Only a single antigenic type of equine adenovirus (EAdV1) has been isolated from horses with respiratory disease [1,2]. EAdV1 occurs worldwide and causes acute upper respiratory tract disease (Fig. 1), conjunctivitis (Fig. 2) and bronchopneumonia (Fig. 3) [2] and infection of the gastrointestinal tract (Fig. 4) leading to the production of soft feces [3]. EAdV1 is peculiarly associated as a dominant pathogen in the uniformly fatal, inherited disease syndrome, primary severe combined immunodeficiency disease (PSCID). When PSCID was first recognized in the early 1970s it was estimated that it caused the death of about 3% of all purebred Arabian foals. Foals are born with a total absence T and B lymphocytes. The disease is inherited as an autosomal recessive gene [4,5]. A consistent and dominant feature PSCID is an inexorably progressive EAdV1 bronchopneumonia (Fig. 5); the virus also causes pathology in a wide variety of organs and tissues including the gastrointestinal tract, liver, pancreas and bladder.
A second serotype, EAdV2 has been isolated from diarrheic foal feces [6].

**Biophysical and Genomic Properties of Equine Adenoviruses**

The biophysical properties of EAdV are similar to those of adenoviruses of other species. EAdV are non enveloped, 70 - 80 nm in diameter and the capsid is composed of 252 capsomers: 240 hexamers occupy the faces and edges of the 20 equilateral triangular facets of an icosahedron and 12 pentamers occupy the corners (Fig. 6a). From each pentamer projects a fiber that for EAdV1 is 50 nm in length with a terminal knob (Fig. 6b); this is the longest fiber observed for any adenovirus [7].

Virions are composed of ten structural proteins ranging from 5 to 120 kilodaltons (K) some of which are present in low abundance and are associated with either the capsid or with the inner core. The inner core contains the double-stranded DNA genome, which for EAdV1 is 34.4 kilobase pairs in length. The genome of adenoviruses has inverted terminal repeats and a 55K protein is covalently linked to each 5' terminus. If the DNA is melted, both strands form single stranded circles. Adenovirus DNA alone is infectious.
which translated into 913 amino acid residues. Similar to other members of the genus *Mastadenovirus*, the EAdV1 hexon gene contains two highly conserved regions at the N- and C-termini, which flank intermediate variable and hypervariable regions. The majority of the residue differences between EAdV1 and other adenovirus hexons occurred in two loops, L1 and L2 that are known for other adenoviruses to protrude from the surface of the capsid. Amino acid comparisons with other adenovirus hexons revealed highest homology with human adenovirus 12 hexon with 72% identical and 83% functionally similar residues, followed by bovine adenovirus 3 hexon with 71% identical and 82% functionally similar residues. Phylogenetic analysis indicates that EAdV1 evolved separately from other adenoviruses (Fig. 7).

Figure 7. Unrooted phylogenetic tree inferred from the nucleotide sequences of the hexon genes of EAdV1 and EAdV2 and 14 other adenoviruses of the genus *Mastadenovirus*. The data was processed with the program DNAML (PHYLIP package). The branches were highly significant (p<0.01) and their lengths are equivalent to the evolutionary distance between the viruses [9,10]. Key: AAdV, avian adenovirus; BAdV, bovine adenovirus; EAdV, equine adenovirus; HAdV, human adenovirus. - To view this image in full size go to the IVIS website at www.ivis.org . -

Nucleotide sequence data for the EAdV2 genome corroborated at the molecular level that EAdV2 is distinct from EAdV1 [9]. The complete sequence of the genes encoding the hexon and the 23K proteinase and the partial sequence of genes encoding E1B/19K, IVa2, DNA polymerase, terminal protein, pVI, DNA binding and 100K proteins were determined. The nine EAdV2 genes appeared to be in the same relative order as homologous genes of other adenoviruses. The EAdV2 hexon was more closely related to the human adenovirus 48 hexon with 71.6% identical and 82.7% functionally similar amino acids than to the EAdV1 hexon gene with which there was 69.3% identical and 80.7% functionally similar residues. The deduced amino acid sequence of the EAdV2 23K proteinase gene was most closely related to bovine adenovirus 3 23K proteinase with 59.7% identity and 75% similarity. Phylogenetic analysis of the hexon and 23K proteinase genes indicated that EAdV2 evolved separately from EAdV1 and other adenoviruses (Fig. 7).

An adenovirus-associated virus was isolated from a foal with respiratory disease following inoculation of equine cell culture [11].

**Cultivation and Replication**

EAdV1 and EAdV2 are highly host cell specific and have been cultivated only in cells of equine origin. As for other adenoviruses the replication cycle of EAdV begins with the attachment of virus to cellular receptors via the penton fibers. Entry into the cell occurs via invagination of clathrin-coated pits. The pentons are removed in the cytoplasm and the core migrates to the nucleus; the genome is released and enters the nucleus. In the nucleus the genome is transcribed according to a complex program. In general adenovirus RNAs are transcribed from five separate regions, situated on both strands of the DNA. The RNA transcripts are spliced, and then translated into about 12, mainly nonstructural, early proteins. Viral DNA replication utilizes the 5'-linked 55K protein as primer and proceeds from both ends by a strand displacement mechanism. Following DNA replication, late mRNAs are transcribed; these are translated into the virus structural proteins, which are made in considerable excess. Virions are assembled in the nucleus, where they form crystalline aggregates (Fig. 8a, Fig. 8b, Fig. 8c and Fig. 8d). By light microscopy intranuclear inclusion bodies (See Fig. 10c) are a prominent feature of adenovirus infected cells. Host cell macromolecular synthesis is progressively shut down during the second half of the replication cycle. Virions are released by cell lysis.

Figure 8a. Thin section of a cultured uninfected equine fetal kidney (EFK) cell. NU = nucleolus. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 8b. Thin section of a cultured EFK cell infected with EAdV1. Note the presence of paracrystalline arrays of adenovirus particles and of other precursor material that represents the newly synthesized viral proteins and nucleic acids. The mass of virus synthesized nucleic acids and proteins present in the nucleus of the infected cell is also the mass that forms the characteristic eosinophilic/basophilic intranuclear inclusion bodies as seen by light microscopy e.g., see Fig. 10c. The "halo" that separates the nuclear membrane and marginated chromatin from the inclusion body in light microscopy (Fig. 10c) is a fixation shrinkage artifact and in not a feature of the thin section electron microscopic image shown in this figure. - To view this image in full size go to the IVIS website at www.ivis.org . -
Antigenic Properties

Shared antigenic determinants are associated with the inner part of the hexamer and these determinants define the two genera *Mastadenovirus* (mammalian) and *Aviadenovirus* (avian). There are also genus-specific determinants on the pentamer. Adenoviruses are typed on the basis of serum neutralization (SN) assays. Type-specific antigenic determinants that are defined by SN and hemagglutination inhibition (HI) assays, are located on the outward-facing surface of the hexamers. The fiber contains type-specific determinants detected by SN assays. Although the fiber binds to specific cellular receptors during adsorption, antibody to the fiber or fiber-pentamer complex is only weakly neutralizing. Most adenoviruses hemagglutinate appropriately chosen red blood cells and HI assays are used for antibody detection. Hemagglutination is mediated by the knob-like tip of the penton binding to receptors on the red blood cell surface.

EAdV1 possesses the common group specific *Mastadenovirus* antigen [12]. HI antibody to EAdV1, as required by definition, is type specific [6,12]. Extensive analysis of adenoviruses recovered from horses with respiratory disease, including PSCID Arabian foals indicated that by SN and HI assays, all were a single antigenic type, designated EAdV1 [1,13]. A second EAdV, designated EAdV2 was unrelated by SN to EAdV1 [6]. EAdV2 does not hemagglutinate human O, *Rhesus* macaque or equine red blood cells so that HI assays have not been developed [6].

Clinical Disease

Many authors have recorded the isolation of EAdV1 from nasopharyngeal swabs obtained from horses with respiratory disease [11,14-22]. Clinical signs include nasal discharge (Fig. 1), coughing after exercise and enlarged submandibular lymph nodes and the delivery of soft feces. Powell et al., [3] reported a study in which 19 horses that had respiratory disease seroconverted to EAdV1; 13 of the 19 horses were 2 years old. Powell et al., also reported the isolation of EAdV1 from 3 horses in a racing stable where there was a history of poor performance. Severe or fatal bronchopneumonia has been occasionally recorded in non immune deficient Thoroughbred foals [15,19].

Respiratory disease signs in an experimentally infected, specific-pathogen-free, 50 day old foal that was cesarean section derived, colostrum-deprived and artificially reared in an EAdV free environment were described [17]. The foal was healthy and 44 days old when infected and had been shown to be immunocompetent by producing high titer bacteriophage neutralizing antibody following experimental inoculation and to produce a delayed type hypersensitivity response in the skin following application of dinitrochlorobenzene as a correlate of cell mediated immune competence. Following intranasal infection with EAdV1 clinical signs included mucopurulent nasal discharge (Fig. 1), severe follicular conjunctivitis, (Fig. 2), transient anorexia and pyrexia and sustained polypnea. There were no changes in blood leucocyte numbers. EAdV1 was readily isolated from nasal, conjunctival and rectal swabs and from lung, trachea, bronchial lymph nodes and small intestine tissue homogenates obtained following an elective postmortem at 6 days post infection.

The clinical response to experimental infection of a 4 day old foal that received colostrum but was artificially reared from 12 hours after birth in an EAdV free environment and which had a 1:320 SN antibody titer and a 1:40 HI antibody titer was similar to foal 1 except that fever was not as marked or as sustained, the conjunctivitis was less severe and nasal discharge was minimal [17]. EAdV1 was readily isolated from nasal and conjunctival swabs and from trachea and lung but not from rectal swabs, small intestine or bronchial lymph nodes when an elective postmortem was conducted 6 days post infection. The production of soft feces in adult horses, indicative of gastrointestinal infection, has been reported as a sole manifestation EAdV infection [3]. Replication of EAdV1 in cells of the gastrointestinal tract was confirmed following experimental intranasal infection (Fig. 4) [17]. EAdV1 was reported as a potential cause of abortion in mares [23]. The latter was reproduced experimentally after intruterine inoculation of the virus [23] but claims for the natural occurrence of EAdV
abortion are unsubstantiated. EAdV2 was isolated from foals with severe diarrhea in which rotavirus was also present [24]. Several of these foals died and at postmortem one had intussusception.

**Pathogenesis**
EAdV1 is particularly recognized in conventional foals as a cause of respiratory disease and conjunctivitis and is presumably frequently acquired as droplet or close contact respiratory or ocular infection. The virus replicates in epithelial cells throughout the respiratory tract producing lysis and sloughing of these cells and a hyperplasic response in underlying uninfected cells (Fig. 9, Fig. 10a, Fig. 10b and Fig. 10c). Respiratory disease in foals is more severe and more likely to be associated with pneumonitis where there is total or partial failure of maternal antibody transfer (Crawford, TB. Personal Communication, 1992).

**Pathology**
Following experimental infection of a colostrum-deprived specific-pathogen-free foal there was gross and histopathological evidence of rhinitis (Fig. 1 and Fig. 9), conjunctivitis (Fig. 2), tracheitis and pneumonia (Fig. 3, Fig. 10a, Fig. 10b and Fig. 10c); the pneumonia was found at an elective postmortem, 6 days post infection. There was both bronchopneumonia and interstitial pneumonia in affected areas of lung. Duodenal villous atrophy (Fig. 4) and idiopathic glomerular hyperplasia were also observed. EAdV antigen was detected by indirect immunofluorescence antibody staining of trachea and lung but not in frozen sections of bronchial lymph node or small intestine. In a specific-pathogen-free foal that received colostrum gross and histological evidence of EAdV1 disease was in general similar but less severe than that observed in a SPF colostrum deprived foal [17].

**Immunity**
Immune competent foals develop significant EAdV1 antibody, recover spontaneously from the disease and generally do not possess recoverable virus by day 10 after exposure [23]. In experimental infections of colostrum-deprived and colostrum-fed foals it was noted that the colostrum-fed foals have less severe changes than colostrum-deprived foals [17,23]. Evidence suggests a high frequency of natural reinfection of susceptible horses, especially in closed populations [21,25]. Reinfection by EAdV1 was found to occur frequently in a group of 16 mares and foals and it was noted that many of these
infections (or reinfections) occurred in the presence of high levels of circulating antibody [21,27]. These authors suggested that since, in humans, infection with adenovirus or rhinoviruses (common cold viruses), IgA in nasal secretions is responsible for resistance to reinfection, a similar situation could occur in horses when rapidly declining levels of nasal antibody following an infection could soon render the horse susceptible to reinfection, despite high serum antibody levels. After intramuscular immunization with live EAdV1 and subsequent development of high serum antibody levels a SPF foal proved resistant to intranasal challenge with EAdV1. Clinical disease did not develop and virus was not isolated, although there was a >2 fold increase in SN antibody after challenge [17]. Immunity was correlated with prior exposure to the virus and high circulating SN antibody levels.

An inactivated EAdV1 vaccine was shown to elicit high antibody titers in rabbits, mice and foals. Using nude mice as a model of T-cell immunodefiency, it was shown that production of EAdV1 SN antibody and, to a lesser extent HI antibody was T lymphocyte dependent [28]. As a measure of cell mediated immune responses specific EAdV1 in vitro lymphocyte blastogenesis (LB) was evaluated, using lymphocytes from 4 vaccinated and 2 control horses. Horses were vaccinated on days 0 and 14, and the LB assays were conducted on days -4, 0, 3, 7, 10, 14, 17, 21, 24 and 28. Lymphocytes from horses were incubated for 4 days with 2 concentrations of inactivated, concentrated and purified EAdV1 antigen. The LB responses for the 2 control horses showed no significant changes during the study period (maximum stimulation indices to EAdV1 antigen for individual horses were between 2.8 and 3.6). The 4 vaccinated horses showed marked increases in stimulation indices in response to EAdV1 antigen (maximum stimulation indices, between 5.3 and 18.6). In control assays, identical lymphocyte preparations from all horses responded normally to phytohemagglutinin [27].

**Diagnosis**

Virus isolation from nasopharyngeal and conjunctival swabs during the acute phase of infection is possible but not frequently reported. EAdV1 may also be isolated from rectal swabs but would need to be differentiated from EAdV2. Detection of adenovirus in negatively stained fecal preparations by electron microscopy is readily achieved. Immune precipitation, complement fixation, hemagglutination, HI and SN assays have been extensively used in diagnosis and seroepidemiologic studies. EAdV1 hemagglutinates human blood group O and equine erythrocytes but not those of sheep or chicken [12]. Based on sequence data to the hexon genes [9,10] reasonably well validated, discriminating diagnostic polymerase chain reactions have been developed for both EAdV1 and EAdV2 [33]. EAdV2 does not to hemagglutinate human O, Rhesus macaque or equine erythrocyte [6] and accordingly HI assays for EAdV2 have not been developed.

**Epidemiology**

Based on serology EAdV1 is worldwide in distribution with incidence rates varying from <2% to 100% depending on the serological test used and/or age, breed, activity and size of the population sampled [1,18,21,29-32,34,35]. In one survey, the prevalence of antibody to EAdV1 increased with age such that ~70% of yearlings and two-year old horses had EAdV1 antibody; this incidence was similar to that of older horses [21].

All adenovirus infections appear to be followed by a latent carrier status and shedding of the virus. Studies in the Pirbright pony herd indicated that EAdV1 infections were often subclinical and virus may persist in the horse for long periods; EAdV1 was recovered from nasopharyngeal swabs up to 68 days after exposure [25].

EAdV1 is presumably endemic in most equine populations. EAdV1 was isolated from a foal, without clinical signs at the time of isolation, at 3 days of age [22]. It seems that foals may acquire infection from their dams or other horses in their cohort during the suckling period even in the presence of detectable levels of maternal antibody [1]. Thus, if there is persistent or repeated EAdV infection, the passive immunity of the foal would be converted to an active immunity, probably without significant clinical disease. Disease would occur if primary infection occurred after maternal antibody had declined, or if the foal was unable to produce an active immune response. Serological evidence indicates a high infection rate in the first year of life, but in some populations 50% of horses <1 year lack EAdV antibody and are presumably at risk to EAdV infection and disease [1].

EAdV2 has been isolated in Australia and in New Zealand [6,36]. Serum from 327 horses, collected from widely separate geographic areas within Australia and from horses of diverse breeds and age, were tested for EAdV2 SN antibody; 77% of these sera were positive (maximum titer 1:640). Nine of 12 (75%) other sera collected from Thoroughbred horses soon after importation to Australia from the United Kingdom were also positive for EAdV2 SN antibody. Of the 339 sera tested in this survey 86% also contained antibody to EAdV1. Seventy of the 339 sera contained antibody to one EAdV type but not the other, and of these 51 were positive for EAdV1 and 19 were positive for EAdV2 [6].

**EAdV1 in Arabian Foals with PSCID**

In a paper titled "Suspected adenovirus bronchitis in Arab foals" [37] it was stated: "In September 1963, a foal was seen with what appeared to be a simple upper respiratory tract infection. It failed to respond to treatment, developed intractable dyspnea and died". This was one of 3 foals described by Johnston and Hutchins [37] that died in 1963, 1965 and 1966 respectively.
Each of the foals had adenoviral bronchopneumonia, one had severe diarrhea and one was shown to be lymphopenic (255 cells/mm³ - mean of 3 hemograms taken 1, 2 and 4 days before the foal died). The foals were sired by a stallion subsequently shown to be a carrier of the defective gene that interferes with development of the immune system and were born to 2 mares also known to be related to known carrier mares and stallions [5].

The same syndrome was subsequently recognized in the United States [38,39] and referred to as adenoviral bronchopneumonia. McGuire and Poppie [4] described additional cases in the United States and presented evidence that the adenoviral bronchopneumonia was incidental to a combined immunodeficiency (called in this review PSCID). Thompson et al., [5] in a retrospective study, described 17 additional cases in Australia and presented evidence that the immunological defect was inherited as a simple, autosomal recessive gene. For other reviews of PSCID see [2,40,41].

Affected foals appear normal at birth and suckle normally. The onset of illness at about 25 days of age (range 14 to 46 days) is marked by a bilateral nasal discharge that is initially clear but subsequently becomes yellow and slimy and may partially occlude the nostrils and make suckling difficult. There is loss of weight, intermittent temperature rises, coughing and dyspnea. The hair coat becomes dry and unkempt and there is frequently loss of hair particularly around the muzzle, eyes and base of the ears. *Dermatophilus congolensis* was identified in some foals with alopecia and dermatitis. The variable age of onset of clinical disease is considered in part related to the variable amount of specific maternal antibody to the various bacterial, protozoal and viral agents that successively infect these foals. A consistent and predominant clinical feature of most identified affected foals is a progressive intractable EAdV1 bronchopneumonia. Intermittent diarrhea is common. Although it was the peculiar and dominant role of EAdV1, particularly in causing bronchopneumonia that drew attention to the syndrome many other pathogenic and commensal microorganisms have been identified as contributing to the final demise of PSCID foals.

Foals with PSCID show a progressive decline in health despite intensive therapy. Antibiotics and supportive therapy, including the administration of fluids and of serum, known to contain high levels of EAdV1 immunoglobulin, seemingly prolong the course of the disease but invariably affected foals die on average at 49 days of age (range 34 to 77 days).

**Gross Pathology and Pathogenesis in PSCID Foals**

The most consistent and obvious gross pathological findings include extensive rhinitis, tracheitis and bronchopneumonia (Fig. 5 and Fig. 11). Typically about 25% of the total lung volume, particularly in the anterioventral portions of the lung, is consolidated and purple in color.

Throughout the lungs, however, there are patches (lobules) of discoloration, which are lower than surrounding lobules. The remaining lung tissue though normal in color is markedly emphysematous and distended, and fails to collapse. Many bronchi particularly in the ventral parts of the lung are plugged with thick, cream-colored exudate. Abscesses from a few mm diameter to several cm diameter, from which *Rhodococcus equi* can be readily isolated in pure culture, are frequent in Australian PSCID foals. McChesney et al., [38] recorded the occurrence in some foals of gray-white foci, 2 - 5 mm diameter, randomly scattered throughout the hepatic parenchyma and of smaller (0.1 mm diameter), similar foci beneath the capsule of the pancreas.

The thymus is small and difficult to find (Fig. 12a and Fig. 12b) the visible remnants of a thymus may be seen as a few scattered islands of salmon-pink colored tissue in an otherwise vacant and completely transparent anterior mediastinum. Lymph nodes throughout the body are small.

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**Figure 11.** Lung of 63 day old pure bred Arabian foal that had primary severe combined immunodeficiency disease (PSCID). There is very extensive bronchopneumonia. The lungs have failed to collapse. On the cut surface of the lung, essentially all bronchi are plugged with thick, cream coloured exudate. Courtesy K.G Johnston, The University of Sydney. - To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 12a.** Gross appearance of the anterior mediastinum of a normal 50 day old foal with the ribs removed showing the thymus which weighed ~350 g. - To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 12b.** Same preparation of a PSCID foal showing the lack of thymic development. - To view this image in full size go to the IVIS website at www.ivis.org.
Histopathology in PSCID Foals
Conjunctivitis, extensive rhinitis (Fig. 13) and tracheitis are present and adenoviral inclusion bodies are readily demonstrated in epithelial cells of these surfaces. There is marked proliferation of bronchiolar epithelium and in the more peripheral of these proliferated cells adenoviral inclusion bodies are readily demonstrated (Fig. 14).

Figure 13. Turinate epithelium of a PSCID foal. Note proliferation and disorientation of cells in the normally columnar ciliated epithelium (lower), the subepithelial inflammatory cell infiltrate and the highly cellular exudate in the lumen (upper). - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 14. Bronchiolitis in PSCID foal showing marked proliferation and disorientation of the bronchial epithelium. Adenoviral inclusions are readily demonstrable (arrow) in the more peripheral of the proliferated cells. The bronchial lumen is filled with necrotic exudate. H and E: x200 [41]. - To view this image in full size go to the IVIS website at www.ivis.org . -

By indirect immunofluorescence the presence of adenoviral antigen in bronchial epithelial cells is demonstrable. Areas of interstitial pneumonia surround affected bronchi. The inflammatory cells infiltrating foci of R. equi abscessation are almost exclusively polymorphonuclear leucocytes; occasional macrophages but no lymphocytes are present. Pneumocystis carinii (Fig. 15a and Fig. 15b), a protozoan that is commonly found in the lungs of humans with HIV/AIDS, is found in the lungs of about half the PSCID foals [42]. Invasive and damaging microflora in PSCID foals is very variable and complex such that no two cases will be the same. In addition to those specific pathogens mentioned herein Mair et al., [43] identified coronavirus and cryptosporidium in the intestinal tract of a PSCID foal.

An outstanding histological finding, first described in 1 of 2 foals examined by McChesney et al., [38] and present in 3 of 5 foals examined by Studdert [41], was an extensive and severe adenoviral pancreatitis (Fig. 16a, Fig. 16b, Fig. 16c and Fig. 16d). The initial lesion appears to be centered on cells lining the acini and to a lesser extent the ducts. Islet cells appeared to be spared initially though subsequently with extension from the initial sites they become involved in the spreading inflammatory response. The inflammatory cells are almost exclusively polymorphonuclear neutrophils. The interlobular septa are greatly thickened and fibrotic (Fig. 16b). Adenoviral inclusion bodies are readily demonstrated in acinus and ductal cells (Fig. 16d) and by indirect immunofluorescence adenoviral antigen was shown to be widely distributed throughout the pancreas. McChesney et al., [38] also recognized a striking adenoviral sialoadenitis involving labial and parotid salivary glands.
None of the foals examined by Studdert [41] had hepatitis although McChesney et al., [38] recognized hepatitis in some PSCID foals. McChesney et al., [38] described the lesions as focal areas of hepatic necrosis; inclusion bodies were not, however, present in the lesions.

Adenoviral cystitis and inflammation of the renal pelvis originally described by McChesney et al., [38] were also common in foals examined by Studdert [41] (Fig. 17a and Fig. 17b).

Changes in the lymphoid tissues were described in detail [41,44]. Histologically most of the thymus is represented by fatty tissue; there is a total absence of cortical lymphocytes (Fig. 18a and Fig. 18b).

Islands of cells that resemble thymic medulla and include Hassall's corpuscles are present in some sections. In the spleen lymphocytic follicles (B lymphocytes) and periarteriolar lymphocytic sheaths (T lymphocytes) are absent (Fig. 19a and Fig. 19b). Lymph nodes are small, follicles (B lymphocytes) are absent and the interfollicular and deep cortical areas (T lymphocytes) are depleted of lymphocytes (Fig. 20a and Fig. 20b).
Clinical Pathology and Diagnosis in PSCID Foals

Foals that were studied by Studdert [41] were all older than 5 weeks and severely ill. None survived longer than 2 weeks after diagnosis. The key to antemortem diagnosis is lymphopenia. Foals had from 0 to about 500 lymphocytes/mm³. It is suggested that counts of less than 1000 lymphocytes/mm³ be considered diagnostic. Affected foals would be expected to be lymphopenic at birth.

Immunoglobulin M is synthesized by the equine fetus [4] and the absence of IgM from presuckle blood would support a diagnosis of PSCID. Postsuckling, IgG, IgM and IgA are demonstrable in foal serum. As these immunoglobulins are removed they are not replaced, thus PSCID foals become hypogammaglobulinemic and probably from 25 days IgM will be absent from their serum.

In vitro measurements of lymphocyte function such as stimulation by phytohemagglutinin, which measures both T and B lymphocytes function are very low [45]. Complement levels and functions are apparently normal [45] as are numbers and functions of monocytes and polymorphonuclear cells [46] although the latter cell types are frequently elevated because of infection, particularly in the terminal stages of the disease.

In the absence of a specific diagnosis a wide spectrum of antibiotic and symptomatic treatments of PSCID foals, were undertaken at great cost but such treatments whilst they may result in temporary remissions are unjustified and in vain. In experimental studies reconstitution of the immune system by bone marrow transplantation [47-50] and transplantation of other tissues [51] have been attempted. The failure of a well matched sibling bone marrow transplantation [49] was attributed to a lack of knowledge of the adverse effects of cytotoxic drugs, especially cyclohexamide in causing renal toxicity, used to counteract graft vs. host reactions and to electrolyte imbalance especially hypokalemia. The protocols for the use of the immunosuppressive cytotoxic drugs were based on human protocols that were clearly inapplicable, because of greatly enhanced cytotoxicity in the horse. Most attempts at transplantation have been unsuccessful with the notable exception of the cases reported by Perryman et al., [50]. Such reconstitution attempts are completely unjustified ethically other than for experimental purposes unless accompanied by castration since they would result in the survival of a homozygous carrier. Control of PSCID in Arabian foals can be achieved by avoiding breeding heterozygous carrier mares and stallions and this is now greatly facilitated by the availability of a carrier test (VetGen www.vetgen.com/scidref1.html).

Inheritance of PSCID

PSCID foals have thus far been recognized only in pure or part bred Arabian foals. The defect is inherited as a simple autosomal recessive gene [5,52]. The genetic defect in PSCID foals has been identified [53-56]. The development of the antigen (epitope) specific receptors on the surface of B and T lymphocytes i.e., surface immunoglobulin (slg) and T cell receptors (TCR) respectively, requires rearrangement by somatic recombination of the V, D, J genes of immunoglobulin heavy chains, the V, J genes of the light chains and the α/β, γδ genes of the TCRs. The enzyme required for these recombination events, generally referred to as the recombinase complex, consists of several components including two endonucleases called RAG1 and RAG2 (for recombination-activating gene) which cut the DNA which is then repaired by several other components including a DNA dependent protein kinase catalytic subunit (DNA-PKcs). It is this later gene that
is defective in one chromosome of carrier mares and stallions and in both chromosomes of PSCID foals. There is a frame shift mutation associated with a five nucleotide deletion common to all affected and carrier horses. Horses with one copy of the gene appear normal, while horses with two copies of the gene manifest PSCID. Oligonucleotide probes against the normal and defective forms of DNA-PKcs are available and these are used for diagnosis (genotyping) of normal, heterozygous (carrier) and PSCID horses.

It was estimated that about 2 to 3% of all pure bred Arabian foals in the United States [52] and Australia [41] were born PSCID which corresponded to a carrier rate of ~30%. This is remarkably high rate for a uniformly lethal defect. There is clear evidence that high prevalence is due to a founder effect dating from ~1880s, probably also to heterozygote advantage and to other influences. More recent data from testing 250 randomly selected Arabian horses in the United States indicated that the frequency of PSCID gene carriers was 8.4% (21/250) which would coincide with 0.18% (1 in 567) Arabian foals affected with PSCID where there is random breeding of the population [57]. With appropriate testing this downward trend in the incidence of PSCID should continue.

Coda
In a general sense adenoviruses are considered mild or relatively inconsequential pathogens. So much so that in both human and veterinary medicine they have been much favoured and promoted as vectors for the delivery of foreign genes in the context of genetically engineered vaccines and for the correction in humans of defective genes. Some of these efforts have resulted in untoward and unexpected outcomes [60]. In normal animal populations the respiratory and gastroenteric disease caused by adenoviruses presumably pass without much attention or specific confirmatory etiologic investigation. The studies reported in this account of equine adenoviruses, particularly those experimental studies in SPF foals, remind us in a pure and uncomplicated form, that adenoviruses are significant pathogens. In conventional foals, even though the virus is cleared within a week or so of infection opportunistic secondary bacterial infection would complicate and prolong the respiratory and enteric disease initiated by the adenoviruses. EAdV1 plays a remarkable and dominant role of in contributing to the death of immune deficient (PSCID) foals. These observations suggest a need for caution in the promotion of adenoviruses as generally insignificant pathogens and hence their suitability as genetically engineered vectors for foreign gene delivery.

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