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CASE REPORT

See page 30 for corresponding Satellite Article

Combined immunodeficiency in 3 foals

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INTRODUCTION

Combined immunodeficiency (CID) is a fatal, inherited disease of Arabian foals first reported in 1973 (McGuire and Poppie 1973). CID is an autosomal recessive condition that results in the absence of both B and T lymphocytes. Affected foals suffer from a variety of infectious disease, and are largely dependent on passively transferred maternal immunity for protection.

Diagnosis of combined immunodeficiency is based on 3 criteria: (1) persistent lymphopenia, (2) absence of IgM in foals over 4 weeks of age, and (3) lymphoid hypoplasia of the thymus, spleen and lymph nodes. This report describes 3 cases of CID presented to the School of Veterinary Medicine, University of Wisconsin-Madison between May and August of 1992.

CASE 1

CASE HISTORY

A 31-day-old Arabian filly foal was presented with a chronic pneumonia of 20 days' duration and a persistent fever of > 39°C. The condition had not responded to treatment with procaine penicillin G (1.5 units x10^6 im, twice/day) for the previous 14 days and trimethoprim sulphamethoxazole (15 mg/kg, po twice/day) for the previous 8 days.

CLINICAL EXAMINATION

The foal was in good body condition and weighed 78 kg. There was a bilateral mucopurulent nasal discharge and tachypnoea (64 breaths/min), with marked abdominal expiratory effort. Rectal temperature was 38.3°C and pulse rate 108/min. Mucous membranes were cyanotic. Thoracic auscultation revealed bilateral inspiratory crackles and inspiratory and expiratory wheezes throughout the lung fields.

CLINICO-PATHOLOGICAL AND RADIOGRAPHIC FINDINGS

Initial diagnostic procedures to evaluate the signs of respiratory disease in this foal included a complete blood cell count (CBC), arterial blood gas evaluation, cytological and bacteriological examination of a trans-tracheal lavage specimen and thoracic radiographs. Abnormal CBC results (Table 1; Day 1) included severe lymphopenia, hyperfibrinogenaemia and a slight left shift. The foal was hypoxaemic (PaO_2 41.5 mmHg) and thoracic radiographs showed signs of a marked generalised interstitial pattern. Cytological examination showed a suppurative neutrophilic exudate in the tracheal fluid, and *Streptococcus zooepidemicus*, sensitive both to penicillin and trimethoprim-sulphamethoxazole, was isolated in culture.

DIFFERENTIAL DIAGNOSIS AND CLINICAL COURSE

Differential diagnoses included causes of infectious pneumonia. The chronic non-responsive nature of the condition, despite antibiotic therapy, suggested the possibility of chronic pulmonary abscessation caused by *Rhodococcus equi* infection. However, the radiographic finding of a predominantly interstitial infiltrate was more consistent with viral infection. Profound lymphopenia suggested that CID should be considered. Serum IgM radioimmunodiffusion (RID) assay was performed to evaluate the foal's immune system; it was anticipated that passively transferred maternal IgM would have become minimal in a foal of this age. This assay required 48 h to perform.

While awaiting the result of the IgM determination, antibiotic therapy was administered (22,000 iu potassium penicillin/kg, iv, 4 times/day, and 15 mg amikacin/kg, iv, once/day). During the next 2 days the foal showed further clinical deterioration. Another CBC showed a persistent lymphopenia (Table 1; Day 2) and serum IgM was undetectable by the RID assay (<12 mg/dl; normal concentration 20–40 mg/dl at 20–40 days of age). These findings supported the tentative diagnosis of CID in this

TABLE 1: Complete blood cell count results

	TWBCC ($x10^9$/litre)	Neutrophils ($x10^9$/litre)	Bands ($x10^9$/litre)	Lymphocytes ($x10^9$/litre)	Monocytes ($x10^9$/litre)	Tot.Prot. (g/litre)	Fibrinogen (g/litre)
Foal 1							
Day 1	5.500	5.115	0.165	0.110	0.110	69	8
Day 2	8.100	7.533	0.081	0.0162	0.324	74	8
Foal 2							
Day 1	6.400	5.696	0.384	0.064	0.192	69	7
Day 2	4.200	3.780	0.420	0	0	70	8
Day 4	4.200	3.864	0	0.042	0.294	n.d.	n.d.
Foal 3							
Pre-referral	10.600	n.d.	n.d.	100	n.d.	n.d.	n.d.
Day 1	16.100	14.490	0.161	0	1.449	62	8
Day 3	11.300	10.057	0.452	0.226	0.565	75	8
Normal range	6.000–12.000	3.000–7.000	0–0.100	1.500–5.000	0–1.000	55–79	2–4

Abbreviations: TWBCC = total white blood cell count; Bands = band form neutrophils; Tot. Prot. = total protein; n.d. = not done.

foal and this, coupled with increasing respiratory distress, prompted the owner to request euthanasia.

AUTOPSY

Gross post-mortem findings included a hypoplastic thymus which was difficult to find within the mediastinal tissue. There was generalised lymphoid hypoplasia. Histological examination showed that the thymic tissue was arranged in islands separated by adipose connective tissue, was depleted of lymphoid cells, contained large numbers of thymic epithelial cells and had no clear differentiation between cortex and medulla. Lymph nodes were extremely hypocellular with no follicular structures in the cortex. In the spleen periarteriolar lymphoid sheaths and germinal centres were absent.

Lung pathology was consistent with a severe diffuse necrotising broncho-interstitial pneumonia, with intranuclear amphophilic inclusion bodies in bronchiolar epithelial cells consistent with adenovirus infection. Adenovirus was subsequently isolated from the lung by tissue culture, and specifically identified by immunofluorescent staining. Within the pancreas there was a necrotising adenitis and intranuclear inclusions were present in ductular epithelial cells, again consistent with adenovirus infection. These post-mortem findings confirmed the diagnosis of CID.

FOAL 2

CASE HISTORY

A 35-day-old Arabian colt foal was presented to the teaching hospital with pneumonia of 5 days' duration. The foal became pyrexic (40^oC) and anorexic the day before presentation and was treated with penicillin and gentamycin.

CLINICAL EXAMINATION

On initial examination the foal was in good body condition (85 kg), but depressed with bilateral mucopurulent nasal discharge. The foal was febrile (40.2^oC), tachycardic (120/min) and tachypnoeic (84 breaths/min) and mucous membranes were cyanotic. Respiration was laboured, with marked abdominal expiratory effort. During thoracic auscultation crackles were heard over the entire lung fields and wheezes over the right caudo-dorsal lung fields.

CLINICO-PATHOLOGICAL AND RADIOGRAPHIC FINDINGS

The foal had a severe lymphopenia, a hyperfibrinogenaemia and a left shift (Table 1, Day 1). Arterial blood gas examination demonstrated respiratory acidosis (pH 7.292, $PaCO_2$ 53.8 mmHg) and hypoxaemia (PaO_2 32.2 mmHg). The transtracheal lavage sample was poorly cellular, but *Rhodococcus equi* was isolated on culture. Thoracic radiographs showed signs of a cranio-ventral bronchopneumonia and a caudo-dorsal interstitial pneumonia (Fig 1); there was no evidence of abscessation.

DIAGNOSIS AND CLINICAL COURSE

This foal was evidently suffering from severe respiratory disease and the radiographic signs were predominantly of interstitial pneumonia. Despite the reportedly short duration of the condition and possible viral aetiology, the profound lymphopenia suggested that CID be considered in the differential diagnosis and therefore a serum IgM RID assay was performed.

Initial medical therapy included ampicillin (12 mg/kg, iv, 4 times/day) and amikacin (15 mg/kg, iv, 4 times/day).

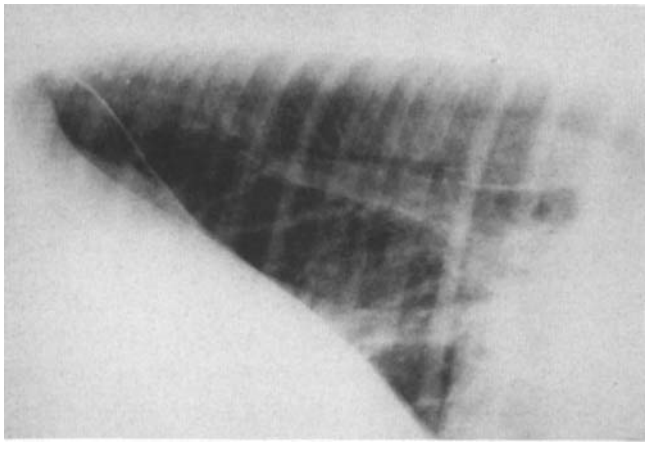

Fig 1: Lateral caudo-dorsal lung field radiograph of Foal 2, demonstrating interstitial pattern of infiltration with no evidence of any abscessation.

No clinical improvement resulted and on Day 3, when the *R. equi* culture result was received the antibiotics were changed to erythromycin (20 mg/kg, po, once/day) and rifampin (10 mg/kg po, twice/day).

Further CBCs on Day 2 and Day 4 showed persistent and severe lymphopenia (Table 1). IgM concentration was initially undetectable (<12 mg/dl) on Day 3 of hospitalisation. These results supported the diagnosis of CID. An intradermal phytohaemagglutinin (PHA) test, which evaluates a delayed-type hypersensitivity T-lymphocyte response was then performed.

Both mare and foal were given intradermal injections of 3 different amounts of PHA (3, 15 and 150 μg, suspended in 100 μl saline) at separate sites in a clipped area over the neck; a similar volume of saline alone was injected at a fourth site. After 24 h there was a doubling of skin-fold thickness (from 2.6 to 5.3 mm) at the injection sites for the 2 higher amounts of PHA for the mare, but no change at any site for the foal. This result further supported the diagnosis of CID and because of continuing deterioration, despite the modified antibiotic regimen, the foal was subjected to euthanasia on Day 5.

Autopsy

On gross examination the thymus was small (6 x 3 x 1 cm) and all lymph nodes were hypoplastic. Histologically the lymphoid tissues were very similar to those in Foal 1, with an absence of cortico-medullary structure in the thymus, or of follicles in lymph nodes. Both tissues were extremely hypocellular. Within the lungs changes were consistent with a chronic, severe bronchointerstitial pneumonia together with a necrotising, proliferative bronchiolitis. Large, amphophilic intranuclear inclusion bodies, typical of an adenovirus infection, were also found in the lungs. There were no lesions typical of *Rhodococcus* pneumonia found at gross or histopathological examination of the lungs, although this organism was again cultured from the lung at autopsy.

FOAL 3

Case history

A 3-month-old Arabian colt foal was presented with a 2-month history of pneumonia that was unresponsive to antibiotic treatment, including an initial 1 week course of oral trimethoprim-sulphamethoxazole, followed by a 1-month course of oral erythromycin and rifampin. Culture of a transtracheal lavage sample 1-week before referral isolated *Proteus vulgaris*. At 3 days before presentation the foal developed pyrexia (40.6°C) and rapid, shallow breathing. A CBC was performed at that time and showed the foal to be severely lymphopenic (Table 1, Pre-referral).

Clinical examination

Although in good body condition (116 kg), the foal was depressed and had a bilateral mucopurulent nasal discharge, severe dyspnoea and tachypnoea (56 breaths/min) with increased abdominal effort on expiration. Crackles were heard over the entire lung fields but were more intense in the cranio-ventral lung fields. Mucous membranes were normal.

Clinico-pathological and radiographic findings

A CBC showed severe lymphopenia, neutrophilia, a slight left shift, monocytosis and hyperfibrinogenaemia (Table 1, Day 1). Thoracic radiographs revealed severe bronchointerstitial pneumonia, suggestive of viral pneumonia. Cytological examination of a transtracheal lavage specimen identified signs of neutrophilic inflammation with increased mucus production. Tracheal fluid bacterial culture yielded *Proteus vulgaris*.

Diagnosis and clinical course

The referring veterinarian was already considering CID in the differential diagnosis at the time of referral and the initial results of our evaluation supported this diagnosis. While awaiting the results of an IgM RID test, the foal was treated with potassium penicillin (22,000 iu/kg, iv, 4 times/day), gentamycin (6.6 mg/kg, iv, once/day), flunixin meglumine (0.5 mg/kg, iv, twice/day) and terbutaline (0.04 mg/kg, po, 3 times/day).

On the 3rd day of hospitalisation the IgM RID result was returned as undetectable (<12 mg/dl). A further CBC documented a persistent lymphopenia (Table 1, Day 3). On the basis of these findings and deteriorating clinical condition the foal was subjected to euthanasia.

AUTOPSY

Changes in lymphoid organs were similar to those described for Foals 1 and 2. The thymus measured 4 x 4 x 3 cm but consisted predominantly of adipose tissue with interspersed islandsof pink poorly defined thymic tissue. Within the lungs there was a necrotising bronchitis and bronchiolitis associated with a chronic, severe bronchointerstitial pneumonia. Amphophilic intranuclear inclusion bodies were present in the bronchial epithelial cells, typical of an adenovirus pneumonia. The pancreas had a chronic, severe ductal adenitis with intranuclear inclusion bodies also consistent with adenovirus infection.

DISCUSSION

The 3 cases described here illustrate common clinical presentations of CID in Arabian foals, being characterised by chronic infectious disease unresponsive to appropriate antibiotic therapy. Adenovirus infection commonly affects CID foals, resulting in an interstitial pneumonia as seen in these cases. Such a presentation accompanied by lymphopenia in an Arabian foal is typically the first indication that CID should be considered in the differential diagnosis. The diagnosis must be carefully substantiated, because if correct it condemns the foal and identifies the sire and dam as carriers.

Diagnosis of CID is made on the basis of 3 criteria (McClure 1990); persistent lymphopenia, absence of serum IgM, and thymic hypoplasia and absence of normal lymph node structure. The first 2 criteria can be identified by ante-mortem tests, but a final diagnosis of CID can be made only on the basis of typical histological findings in lymphoid organs.

Persistent lymphopenia (<1,000 x 10^9 cells/litre) is a consistent feature of this disease. It is important that multiple counts be performed at intervals of several days. Repeating the lymphocyte count is important because a severely ill immunocompetent foal can still become temporarily lymphopenic. To evaluate the humoral arm of the immune system, a serum IgM RID assay can be performed either before the foal sucks colostrum or after 4 weeks of age. CID foals should have no serum IgM at those times, but between these two periods passively transferred maternal IgM may be detected. IgM has a 4–5-day half-life and by 3 weeks of age a foal should therefore have eliminated more than 95% of the maternal IgM antibodies (Riggs 1987), although serum IgM can occasionally be detected up to the age of 30–40 days (L. E. Perryman, personal communication).

TABLE 2: Results of elecrophoresis analysis of serum protein, and RID determinations of serum IgM and IgG concentrations

	Foal 1 (31 days)	Foal 2 (35 days)	Foal 3 (3 months)
Electrophoresis results			
Total protein	69 g/litre	69 g/litre	62 g/litre
Albumen	46%	36%	50%
α1	8%	4%	3%
α2	8%	27%	27%
β1	17%	5%	13%
β2	13%	23%	7%
γ	8%	5%	0%
Patient RID results			
IgG (mg/dl)	870	670	<200
IgM (mg/dl)	<12	<12	<12
Age-matched normal RID results*			
IgG (mg/dl)	480 ± 293	480 ± 293	380 ± 188
IgM (mg/dl)	30 ± 10	30 ± 10	61 ± 22

*Data from Riggs (1987).

Tests commonly used to assess immunoglobulin levels in the newborn foal, such as total protein and serum IgG concentrations, are inappropriate for the diagnosis of CID in a foal. Total protein concentrations are often normal, as in these 3 foals. Electrophoretic examination of serum proteins demonstrated the presence of γ globulins in Foals 1 and 2, but RID assays identified these proteins as IgG (Table 2). These two foals therefore still had significant amounts of maternal passively transferred IgG which, with a half-life of 14–21 days, can still be present at 25–35% of initially transferred levels in 1-month-old animals (McClure 1990). Foal 3 at 3 months of age had no detectable IgG or IgM according to the RID assay, and consequently no γ globulins in the electrophoretic analysis. However, total protein levels in Foal 3 were normal due to elevations of α and β globulins, which were also evident in the other foals. These elevations may represent increased concentrations of inflammatory proteins whose production is unaffected by this condition. These 3 cases therefore demonstrate the utility of IgM assays in providing the earliest and most specific test of the foal's ability to produce immunoglobulins.

Other tests of immunocompetence which assess the lymphoid system can be used to evaluate CID suspects. In the case of Foal 2 an intradermal phytohemagglutinin (PHA) test was used to assess T-lymphocyte function. Such assays are further discussed in the accompanying satellite article (Lunn and McClure, p. 30). As in these foals, there is no deficit in the number or function of neutrophils or monocytes, whose progenitors are unaffected by the defect present in CID foals. Increases in serum fibrinogen in response to infection are similarly unaffected (Table 1).

The third and essential criterion for the diagnosis of CID is histological evidence of typical changes in lymphoid organs. A lymph node biopsy is extremely valuable in the diagnosis of CID but locating the hypoplastic lymph nodes present in CID cases is very difficult. Therefore final diagnosis is usually made *post mortem*. A small or grossly undetectable thymus and small lymph nodes are characteristic gross lesions in CID (Valli 1985). Histologically, the thymus consists of small hypocellular lobules, interspersed with adipose tissue (Foal 2). The lobules have no corticomedullary differentiation and contain epithelial cells and Hassall's corpuscles, with a few widely dispersed lymphocytes (Foal 2). Within lymph nodes,

follicles and germinal centres are absent and the cortex consists of a reticular framework with small accumulations of lymphocytes. Within the spleen the white pulp is absent.

Secondary infectious diseases characteristic of CID foals and are usually the reason the owner seeks veterinary attention. These infections are often caused by agents that are rarely pathogenic in immunocompetent animals. Adenovirus infection resulting in pneumonia is common in CID foals, and the adenovirus can infect other tissues such as the pancreas, as in Foals 1 and 3 in this report. *Pneumocystis carinii* is another opportunistic respiratory pathogen in CID cases. Gastrointestinal infections may be caused by otherwise uncommon pathogens such as coronavirus or cryptosporidia (Mair *et al.* 1990; Bjorneby *et al.* 1991).

CID is an autosomal recessive disease that affects Arabian and Arabian cross bred foals, although sporadic cases have been reported in other breeds (Perryman *et al.* 1984). It is estimated that approximately 2–3% of Arabian foals have CID; this would suggest that 25% of Arabian horses carry the recessive gene (Poppie and McGuire 1977). Currently there is no test to detect carriers, other than costly and impractical multiple breedings with known carriers (Kettler *et al.* 1989). CID foals lack functional B- and T-lymphocytes, whereas neutrophils, macrophages, natural killer cells and the complement system function normally. Prothymocytes are present in the thymus of CID foals but are unable to mature into functional T-lymphocytes (Wyatt *et al.* 1987; Perryman *et al.* 1988b). The rare lymphocytes that are present in the circulation of CID foals are prothymocytes or natural killer cells. The successful immunological reconstitution of one CID foal with a histocompatible bone marrow transplant indicates that the architecture of the thymus and other lymphoid organs is intact (Perryman *et al.* 1988a). This result supports the hypothesis that a biochemical defect in prothymocytes results in their inability to mature into T- and B-lymphocytes.

Most CID foals become ill by 1 month of age and die by 3 months of age. Initially, foals may grow normally but then succumb to chronic infections which are poorly responsive even to appropriate antibiotic therapy. Therefore CID should be considered in the differential diagnosis of any Arabian foal with a chronic, unresponsive infection and which is persistently lymphopenic.

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