Common variable immunodeficiency in three horses with presumptive bacterial meningitis

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A 6-year-old Thoroughbred gelding (horse 1) was referred to the George D. Widener Hospital for Large Animals for evaluation of left hind limb lameness and a suspected pelvic fracture. The horse had been acquired 9 months earlier and was used for pleasure riding. Mild hind limb stiffness had first been noticed 1 month previously and had progressed to grade 4 of 5 left hind limb lameness that was unresponsive to administration of phenylbutazone. In addition, the horse had become anorectic and lost weight.

On initial physical examination, the horse was lethargic but responsive and in poor body condition; body weight was 463 kg (1,020 lb). Generalized muscle fasciculations were seen. Grade 4 of 5 left hind limb lameness was evident, and the stifles and toes were externally rotated. The left gluteal and quadriceps muscles were atrophied. Although the cranial phase of the stride was normal, the limb collapsed during the weight-bearing phase at a walk. There was no obvious joint effusion or signs of pain on palpation or flexion of the limb. Heart rate was 52 beats/min, rectal temperature was 38.3°C (100.8°F), and respiratory rate was 16 breaths/min.

Results of a CBC and serum biochemical profile were unremarkable other than high fibrinogen concentration (778 mg/dL; reference range, 150 to 375 mg/dL). External and transrectal palpation of the pelvis failed to reveal any crepitation or signs of pain. Results of nuclear scintigraphy of the hind limbs, thoracolumbar portion of the vertebral column were unremarkable. The horse was treated with phenylbutazone (4 mg/kg [1.8 mg/lb], PO, q 12 h) for 48 hours, but no improvement was seen. On day 3, the horse was hyperresponsive to tactile and auditory stimuli; appeared to resent palpation of the entire spine, particularly the cervical region; and would not flex or lower its neck. Because of the muscle loss and fasciculations, a possible diagnosis of equine motor neuron disease was considered. However, histologic examination of a biopsy specimen from the sacrocaudalis dorsalis medialis muscle did not reveal any type 1 muscle fiber atrophy.

Serum vitamin E concentration was 3.75 ppm (reference range, 1.5 to 10 ppm). Results of a western blot assay and ELISA for evidence of infection with *Borreli burgdorferi* were negative. Radiographs of the cervical portion of the vertebral column were unremarkable.

Analysis of CSF obtained from the lumbosacral space revealed a high nucleated cell count (810 cells/µL; reference range, 0 to 5 cells/µL) with 87% nondegenerate neutrophils, 9% macrophages, and 4% small lymphocytes. The RBC count was 10 cells/µL, and the total protein concentration was slightly high (121 mg/dL; reference range, < 100 mg/dL). Results of cytologic analysis of the CSF were consistent with supplicative inflammation, but no microorganisms were seen. A sample of CSF and 2 blood samples were submitted for aerobic and anaerobic bacterial culture.

A presumptive diagnosis of bacterial meningitis was made, and empirical treatment with potassium penicillin (124 U/kg/min [56 U/lb/min]) and rifampin (5 mg/kg [2.27 mg/lb], PO, q 12 h) was initiated while results of bacterial culture were pending. Fluixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, q 12 h) and butorphanol (0.02 mg/kg [0.01 mg/lb], IM, q 6 h) were administered for pain management, and a balanced electrolyte solution was administered IV (2.2 mL/kg/h [1 mL/lb/h]) to maintain hydration in the presence of anorexia. Vitamin E (15 U/kg [6.8 U/lb], PO, q 24 h) was administered for its antioxidant properties.

Bacterial culture of the blood samples did not yield any growth, but culture of the CSF yielded light growth of a coagulase-negative *Staphylococcus* spp. Antimicrobial susceptibility was not tested.

Because primary bacterial meningitis is rare in adult horses, the possibility of an immunodeficiency was investigated. Heparinized blood and serum samples were shipped overnight to the Cornell University Department of Clinical Sciences for testing. Isolated peripheral blood lymphocytes were tested by means of flow cytometry for cell surface antigens with a panel of monoclonal antibodies characterized in the First and Second International Workshops on Equine Immunity.
Leukocyte Antigens of peripheral blood lymphocytes revealed B-cell lymphopenia with relative increases in the proportions of T cells (Table 1). Serum IgA, IgG, and IgM concentrations were measured by means of single radial immunodiffusion, and hypogammaglobulinemia was identified (Table 2).

After 1 week of treatment with penicillin and rifampin, clinical improvement was observed. The horse's appetite had returned, and the amount of weight the horse would bear on the left hind limb had increased. Plasma fibrinogen concentration had decreased (430 mg/dL). Intravenous fluid therapy and administration of butorphanol were discontinued. A repeated CBC revealed leukocytosis (13,800 cells/L) with neutrophilia (11,868 neutrophils/L), and fibrinogen concentration had increased to 506 mg/dL. Analysis of a CSF sample obtained 2 weeks after admission revealed a decrease in the nucleated cell count (81 cells/µL) and an increase in the total protein concentration (281 mg/dL). Antimicrobial treatment was empirically changed to oral administration of trimethoprim-sulfamethoxazole (30 mg/kg [13.6 mg/lb], PO, q 12 h) and rifampin. Administration of flunixin meglumine (1.1 mg/kg, IV, q 12 h) was reinstituted. After 1 week of treatment with penicillin and rifampin, clinical improvement was observed. The horse's appetite had returned, and the amount of weight the horse would bear on the left hind limb had increased. Plasma fibrinogen concentration had decreased (430 mg/dL). Intravenous fluid therapy and administration of butorphanol were discontinued. Analysis of a CSF sample obtained 2 weeks after admission revealed a decrease in the nucleated cell count (81 cells/µL) and an increase in the total protein concentration (281 mg/dL). Antimicrobial treatment was empirically changed to oral administration of trimethoprim-sulfamethoxazole (30 mg/kg [13.6 mg/lb], PO, q 12 h) and rifampin. Administration of flunixin meglumine (1.1 mg/kg, IV, q 12 h) was reinstituted. The horse's condition improved in the subsequent 48 hours, and the horse was discharged on day 27. The underlying cause of the left hind limb lameness remained undetermined, but nerve compression by an abscess at the fourth and fifth lumbar nerve rootlets or more distally along the nerve was considered a possibility, as clinical signs resembled femoral nerve paralysis.

Treatment was changed to potassium penicillin (26,000 U/kg [11,800 U/lb], IV, q 6 h) and enrofloxacin (7.5 mg/kg [3.4 mg/lb], IV, q 24 h). Administration of rifampin was continued, and treatment with flunixin meglumine (1.1 mg/kg, IV, q 12 h) was reinstituted. The horse's condition improved in the subsequent 48 hours, and the horse was discharged on day 27. The underlying cause of the left hind limb lameness remained undetermined, but nerve compression by an abscess at the fourth and fifth lumbar nerve rootlets or more distally along the nerve was considered a possibility, as clinical signs resembled femoral nerve paralysis.

**Table 1**—Results of phenotyping of peripheral blood lymphocytes from 3 horses with common variable immunodeficiency.

<table>
<thead>
<tr>
<th>Lymphocyte marker</th>
<th>Horse 1</th>
<th>Horse 2</th>
<th>Horse 3</th>
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<tr>
<td></td>
<td>Initial</td>
<td>2 mo</td>
<td>4 mo</td>
</tr>
<tr>
<td>T cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2+</td>
<td>95.9</td>
<td>93.6</td>
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</tr>
<tr>
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<td>CD11a/CD18</td>
<td>99.9</td>
<td>99.7</td>
<td>99.3</td>
</tr>
</tbody>
</table>

Data are given as percentage of cells positive for each marker. The reference range represents mean ± SD for 6 healthy adult horses. MHC = Major histocompatibility complex.

**Table 2**—Serum immunoglobulin concentrations measured by means of single radial immunodiffusion in 3 horses with common variable immunodeficiency.

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
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<th>Horse 2</th>
<th>Horse 3</th>
</tr>
</thead>
<tbody>
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<td>Initial</td>
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<td>4 mo</td>
</tr>
<tr>
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<td>200</td>
</tr>
<tr>
<td>IgG</td>
<td>280</td>
<td>400</td>
<td>54</td>
</tr>
</tbody>
</table>

Data are given as milligrams per deciliter.
The same antimicrobial treatment was continued until day 51, when treatment was changed to doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h). Peripheral blood lymphocyte phenotyping was performed and serum immunoglobulin concentrations were measured at 2-month intervals, and persistent B-cell lymphopenia and hypogammaglobulinemia were confirmed, despite the improvement in the horse’s clinical condition (Tables 1 and 2). Serum tetanus antibody titers were measured before and 1 month after tetanus toxoid administration by use of a comparative toxin-antitoxin neutralization test in guinea pigs and a competitive ELISA. Both assays revealed a lack of increase in specific antibody titer following vaccination.

Six months after initial examination, additional immunologic testing was performed. Peripheral blood leukocytes were isolated from the horse and from an apparently healthy 8-year-old Thoroughbred mare (control horse) by means of Ficoll gradient centrifugation. For the patient horse, the cell population consisted of 27.2% lymphocytes, 13.3% monocytes, and 51.1% neutrophils, whereas for the control horse, the cell population consisted of 65% lymphocytes, 13.4% monocytes, and 18.3% neutrophils. Isolated leukocytes were cultured in RPMI medium and tested in triplicate (200,000, 100,000, and 50,000 cells/well) with or without addition of pokeweed mitogen (PWM; final concentration, 2.5 µg/mL), phytohemagglutinin (PHA; 5 µg/mL), concanavalin A (ConA; 5 µg/mL), and lipopolysaccharide (LPS; 2.5 µg/mL), as described. Plates were incubated for 3 days at 37°C in 5% CO₂. Cells were pulsed with 0.8 µCi of [3H]-thymidine/well for the last 8 hours of incubation. Well contents were harvested onto glass fiber filters, and [3H]-thymidine incorporation was measured with a liquid scintillation beta counter. The lymphocyte proliferation assay revealed that the patient’s lymphocytes had inferior in vitro responses to mitogens, compared with responses for lymphocytes from the control horse (Figure 1). However, it could not be determined whether this was a result of decreased function of the patient’s lymphocytes or of the lower number of lymphocytes in samples from the patient.

Simultaneous flow cytometric measurement of phagocytosis and oxidative burst activity of isolated peripheral blood phagocytes was performed as described. Briefly, propidium iodide-labeled Staphylococcus aureus (red fluorescent) was used to measure uptake of bacteria by phagocytes. Bacteria were opsonized with pooled serum from healthy control horses or not opsonized. Trypan blue was added to quench fluorescent signals imparted by nonphagocytized bacteria and extracellular bacteria that adhered nonspecifically to the surface of the phagocytes. Oxidation of nonfluorescent dihydro-rhodamine 123 by reactive oxygen intermediates to the green fluorescent rhodamine 123 was measured after phagocytosis of bacteria. Results were collected for both phagocytosis and oxidative burst activity and were expressed as mean fluorescence. For the opsonization assays, bacteria were opsonized with serum or heat-inactivated serum from the patient and results were compared with results for pooled serum from healthy control horses. Phagocytosis and oxidative burst activity of the patient’s phagocytes were comparable to that of the phagocytes from the control horse (Figure 2), and serum opsonization capacity for the patient was comparable to that for the pooled serum from healthy control horses.

A capture antibody ELISA was performed to detect immunoglobulin isotypes in serum from the patient. Goat anti-horse IgG (H+L) was coated onto ELISA plates as the capture antibody for serum immunoglobulins. Murine monoclonal antibodies against equine IgGa, IgGb, and IgG(T) were used to determine immunoglobulin isotypes. Peroxidase-conjugated goat anti-mouse IgG (H+L) was used to detect bound mouse monoclonal antibodies. Serum was tested in 10-fold dilutions ranging from 1:10⁵ to 1:10⁶. Serum concentrations were expressed as optical densities at a 1:1⁰ dilution. Whereas IgGa and IgG(T) concentrations were comparable to concentrations for the control horse, the IgGb concentration was markedly lower in the patient’s serum (Figure 3).

Antimicrobial treatment with doxycycline was continued indefinitely. One year after initial examination, the horse was in excellent body condition and appeared healthy. A left hind limb gait deficit was barely noticeable at a trot 6 months after hospital discharge but eventually resolved, and the horse was used for pleasure riding. Follow-up immunologic testing revealed the same pattern of immunodeficiency.

A 14-year-old Thoroughbred mare (horse 2) that was unrelated to horse 1 was evaluated because of an
acute onset of neurologic deficits shortly after being transferred to a breeding facility. The horse had been vaccinated annually against eastern and western equine encephalitis, tetanus, influenza, equine herpes virus 1, and 4 infection, and rabies. Hind limb ataxia and weight loss of 1 year's duration were reported. Because results of an ELISA for antibodies against B burgdorferi had been positive, the referring veterinarian had treated the horse with doxycycline (10 mg/kg, IV, q 6 h) and rifampin (5 mg/kg, IV, q 6 h) concurrently with supportive treatment (ie, IV administration of crystalloid fluids). However, on day 2, the horse's rectal temperature was 39.7°C (103.4°F), and a neutrophilia (756 lymphocytes/µL), hypoproteinemia (5.4 g/dL; reference range, 2.6 to 4.1 g/dL). Initial CBC revealed marked lymphopenia (370 lymphocytes/µL) and weight loss of 1 year's duration were reported. Results of tests for equine protozoal myeloencephalitis, initial treatment included administration of trimethoprim-sulfamethoxazole (30 mg/kg, PO, q 12 h), pyrimethamine (1 mg/kg [0.45 mg/lb], PO, q 24 h), and flunixin meglumine (1.1 mg/kg, PO, q 12 h).

The horse's condition appeared stable until day 9, when the ataxia became more severe. Cytologic evaluation of a follow-up CSF sample revealed an increase in nucleated cell count (2,200 cells/µL; 82% nondegenerate neutrophils, 19% macrophages, and 9% lymphocytes), total protein concentration (463 mg/dL), and RBC count (230,000 cells/µL). On the basis of these results, a presumptive diagnosis of suppurative bacterial meningitis was made. Bacterial culture of the second CSF sample yielded a coagulase-negative Staphylococcus sp and Sphingobacterium multivorum. Antimicrobial susceptibility of the Staphylococcus isolate was not determined, but the S multivorum isolate was found to be susceptible to chloramphenicol, rifampin, tetracycline, and tetracycline-clavulanic acid. Administration of pyrimethamine was discontinued, and treatment with potassium penicillin (38,000 U/kg [17,000 U/lb], IV, q 6 h) and rifampin (5 mg/kg, PO, q 12 h) was initiated. The clinical signs improved, and the horse was discharged after 6 weeks. Mild (grade 1 of 4) proprioceptive deficits were still present at the time of discharge. Antimicrobial treatment was continued for 1 month after discharge.

The horse was reported healthy until 5 months later when it was transported to a breeding facility. Signs of acute abdominal pain developed, and the horse was referred for evaluation. On initial examination, the horse was in poor body condition and moderately dull. Body weight had decreased to 565 kg (1,245 lb). The horse was afebrile, and results of auscultation of the cardiac, respiratory, and gastrointestinal systems were unremarkable. Results of a CBC and serum biochemical profile were consistent with hyperfibrinogenemia (960 mg/dL) and mild lymphopenia (1,456 lymphocytes/µL).

Routine diagnostic testing failed to identify the source of abdominal pain, and signs resolved spontaneously with supportive treatment (ie, IV administration of crystalloid fluids). However, on day 2, the horse's rectal temperature was 39.7°C (103.4°F), and a CBC revealed marked lymphopenia (370 lymphocytes/µL). Despite extensive diagnostic testing, including abdominal ultrasonography, abdominocentesis, reproductive tract examination, thoracic radiography and ultrasonography, urinalysis, bacterial culture of a urine sample, gastric endoscopy, radiography of the cervical portion of the vertebral column, a polymerase

cell count (17 cells/µL; 70% lymphocytes, 21% nondegenerate neutrophils, and 9% macrophages) and total protein concentration (209 mg/dL). No microorganisms were seen on cytologic examination of the CSF, and bacterial culture did not yield any growth. Results of western blot analysis of serum and CSF for evidence of infection with Sarcocystis neurona were negative. Serum neutralization tests of paired serum samples did not reveal any increase in titer of antibodies against equine herpes virus 1, and results of a West Nile virus serum IgM capture ELISA were negative. Pending results of tests for equine protozoal myeloencephalitis, initial treatment included administration of trimethoprim-sulfamethoxazole (30 mg/kg, PO, q 12 h), pyrimethamine (1 mg/kg [0.45 mg/lb], PO, q 24 h), and flunixin meglumine (1.1 mg/kg, PO, q 12 h).

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chain reaction assay of a buffy coat sample for *Anaplasma phagocytophila*, an immunofluorescent antibody test for *A phagocytophila* and *Brucella* spp, an immunofluorescent antibody test for antinuclear antibodies, a serum neutralization test for equine viral arteritis, and an agar gel immunodiffusion test for equine infectious anemia, the cause of the fever and hyperfibrinogenemia could not be identified. Concentrations of adrenocorticotropic hormone and insulin measured in 3 blood samples collected at 8-hour intervals were within reference limits.

Empirical treatment with potassium penicillin (22,000 U/kg [10,000 U/lb], IV, q 6 h) and gentamicin (7 mg/kg [3.18 mg/lb], IV, q 24 h) was begun. Despite this, the plasma fibrinogen concentration remained high (1,084 mg/dL). On day 4, the horse developed thrombocytopenia (81,160 platelets/µL; reference range, >100,000 platelets/µL) and edema of the distal portions of all 4 limbs. Repeated blood tests revealed persistent lymphopenia with lymphocyte counts ranging from 915 to 1,386 lymphocytes/µL. Hypoproteinemia and hypoglobulinemia (3.3 and 1.85 g/dL, respectively) persisted.

On the basis of the horse's history and the persistent lymphopenia and hypoglobulinemia, an immunodeficiency was suspected. Peripheral blood lymphocyte phenotyping revealed a decrease in the B-cell percentage with relative increases in T-cell percentages (Table 1). Serum concentrations of IgG and IgA were normal, but the serum IgM concentration was undetectable (1). Serum concentrations of IgG and IgA were normal, but the serum IgM concentration was undetectable (Table 2). Serum tetanus antibody titers were measured before and 1 month after tetanus toxoid administration by use of a comparative toxin-antitoxin neutralization test in guinea pigs and a competitive ELISA® and revealed a lack of increase in specific antibody titer following vaccination.

Peripheral blood leukocytes were isolated for proliferation assays. For horse 2, the cell population consisted of 25.8% lymphocytes, 16.4% monocytes, and 49.1% neutrophils. The control cell population was the same as that used for horse 1. For horse 2, lymphocyte responses to ConA, PHA, PWM, and LPS were decreased, compared with responses for the control horse (Figure 2). Phagocytosis, oxidative burst activity, and opsonization capacity were comparable to values for a control horse (Figure 2).

The horse improved, and after 1 week, treatment was changed to trimethoprim-sulfamethoxazole (30 mg/kg PO, q 12 h) and metronidazole (15 mg/kg [6.8 mg/lb], PO, q 6 h). By day 20, the horse’s fibrinogen concentration had decreased (514 mg/dL) and the thrombocytopenia had resolved, but lymphopenia (1,122 cells/µL) persisted. The horse was discharged to the owner's care, and antimicrobial treatment was continued for several weeks. Reportedly, whenever treatment was discontinued, the horse developed a persistent fever. Therefore, antimicrobial treatment consisting of administration of potentiated sulfonamides or doxycycline was continued indefinitely.

Repeated peripheral blood lymphocyte phenotyping and measurement of serum immunoglobulin concentrations indicated persistence of B-cell lymphopenia, low serum IgG concentration, undetectable IgM concentration, and normal IgA concentration. Serum IgGb concentration measured after the onset of IgG deficiency was low, compared with concentration in a control horse (Figure 3).

An 8-year-old Arabian mare (horse 3) with a 10-day history of ataxia and dullness was admitted for examination. The horse had been vaccinated annually against eastern and western equine encephalitis, tetanus, influenza, and rabies. The horse had been treated with phenylbutazone (2.2 mg/kg PO, as needed) and trimethoprim-sulfamethoxazole (30 mg/kg, PO, q 12 h) for 1 week prior to referral, but its condition had continued to deteriorate.

Results of an initial physical examination were unremarkable except for dullness, moderate to severe ataxia of all 4 limbs, and hypermetria of the forelimbs. Body weight was 398 kg (877 lb). Results of initial laboratory tests were within reference ranges. Serum neutralization testing of paired serum samples did not reveal any increase in titer of antibodies against equine herpes virus 1. Results of a West Nile virus serum IgM capture ELISA® were negative. Results of a western blot assay for serologic evidence of *S neorona* infection were weakly positive. Radiography of the skull and cervical portion of the vertebral column did not reveal any abnormalities, and results of endoscopic examination of the pharynx, larynx, trachea, and auditory tube diverticula (guttural pouches) were unremarkable. A sample of CSF obtained by means of lumbosacral puncture was markedly xanthochromic; nucleated cell count (223 cells/µL) and total protein concentration (1.600 mg/dL) were high, and there was marked neutrophilic pleocytosis. No bacteria were seen on cytologic examination of the CSF and bacterial and fungal cultures of the CSF did not yield any growth.

Although results of bacterial and fungal culture were negative, a presumptive diagnosis of bacterial meningitis or brain abscess was made. Treatment included administration of trimethoprim-sulfamethoxazole (30 mg/kg PO, q 12 h), rifampin (5 mg/kg PO, q 12 h), dimethyl sulfoxide (1 g/kg as a 10% solution, IV, q 24 h for 3 consecutive days), and flumixin meglumine (1.1 mg/kg IV, q 12 h). By day 3, the horse's condition had not improved, and mannitol (1.5 g/kg [0.68 g/lb], IV, q 12 h), osmotic therapy (0.1 mg/kg [0.045 mg/lb], IV, q 24 h initially then decreasing to 0.05 mg/kg [0.023 mg/lb], IV, over 4 days) were administered to treat possible cerebral edema. Again, no improvement was noted. A CBC revealed leukopenia (3,700 cells/µL) with lymphopenia (629 lymphocytes/µL), and analysis of a CSF sample collected on day 7 revealed an increase in the nucleated cell count (400 cells/µL) and a decrease in the total protein concentration (545 mg/dL). The horse's neurologic deficits stabilized; however, the horse remained moderately lethargic and ataxic.

On day 21, analysis of a third CSF sample revealed a decrease in nucleated cell count (14 cells/µL) and total protein concentration (145 mg/dL). Serum biochemical testing revealed hypoproteinemia (5.1 g/dL) and hypoglobulinemia (2.59 g/dL). Potassium penicillin (25,000 U/kg [11,363 U/lb], IV, q 6 h) was empirically added to the previously prescribed treatment. Within 48 hours, the horse became more alert and...
Bacterial meningitis in horses.

To our knowledge, been previously reported as a cause of meningitis in horses is Corynebacterium diphtheriae, but clinical reports of S. multiforme have been documented as a cause of meningitis in horses.

The horse was reevaluated 6 weeks later, at which time clinical signs had resolved. Analysis of a CSF sample revealed a low nucleated cell count (8 cells/µL). Results of cytologic examination were normal, and the total protein concentration was within reference limits (79 mg/dL). On the basis of these results, antimicrobial treatment was discontinued.

Two years later, the horse was examined because of bilateral chemosis and blepharospasm of recent onset. The horse had lesions consistent with bilateral chronic uveitis (aqueous flare and a diffuse cataract in the left eye). There were no other clinically important findings on physical examination. A CBC revealed mild leukopenia (5,200 cells/µL) and lymphopenia (1,458 lymphocytes/µL).

Because of the horse's persistent lymphopenia and history of bacterial meningitis, immunologic testing was pursued. Phenotyping of peripheral blood lymphocytes revealed a decrease in the percentage of B cells with relative increases in the percentages of T cells (primarily CD8+ T cells; Table 1). Serum IgG and IgA concentrations were within reference limits, but serum IgM concentration was low (Table 2). Serum IgGb concentration was comparable to concentration in a control horse (Figure 1). The leukocyte population for horse 3 consisted of 24.2% lymphocytes, 17.3% monocytes, and 49.4% neutrophils.

Phagocytosis, oxidative burst activity, and opsonization capacity were comparable to values for a control horse (Figure 2). Despite these findings, except for intermittent episodes of uveitis, the horse remained healthy without additional antimicrobial treatment. Atropine was administered topically and flunixin meglumine was administered systemically as needed to control the uveitis.

Bacterial meningitis is a documented complication of sepsis in neonatal foals6-8 but is considered rare in adult horses. Cryptococcus neoformans,13,15 Streptococcus suis,16 Streptococcus equi,17 Actinomyces spp,18 and Klebsiella pneumoniae19 have been documented as causes of bacterial meningitis in adult horses. However, results of bacterial culture of CSF are often negative, and a presumptive diagnosis is made on the basis of clinical findings and response to treatment.21 Coagulase-negative Staphylococcus organisms are common skin contaminants, and S. multiforme has not, to our knowledge, been previously reported as a cause of bacterial meningitis in horses. Spingobacterium multivorum is a gram-negative bacillus occasionally isolated from human clinical specimens that is characterized by resistance to many antimicrobial agents22 but is only rarely incriminated as a human pathogen.21 Clinical reports22,23 of S multiforme infection in humans have in common the fact that all patients had a systemic immunodeficiency. The clinical importance of the isolates obtained from the CSF from horses 1 and 2 in the present report is questionable. Although sample contamination likely occurred during collection of CSF from horse 2 because of the horse's severe neurologic deficits, these bacteria may have played a role as pathogens in the immunodeficient horses.

Failure of adequate passive transfer of humoral immunity has been recognized as a predisposing factor for sepsis and secondary bacterial meningitis in newborn foals.24,25 Although most human patients with bacterial meningitis are not immunodeficient, the higher incidence of community-acquired meningitis in young children (<2 years old) and elderly individuals (>65 years old) suggests that an age-related lack of adequate immune response plays a role in susceptibility to the disease.26 Congenital and acquired agammaglobulinemia and hypogammaglobulinemia (ie, severe combined immunodeficiency and common variable immunodeficiency [CVID], respectively) are also associated with increased risk of meningitis in people.27 Other epidemiologic factors that increase the risk of meningitis in adults are the presence of an underlying systemic disease, cancer, and HIV infection.28,29 In addition to hypogammaglobulinemia, complement deficiencies (C5, C6, C7, C8, and C9 components) have been associated with a higher prevalence of meningococcal infection in human patients, whereas other immunologic abnormalities (eg, disorders of phagocytic function and IgA deficiency) are rarely associated with bacterial meningitis.27 Although suppressive meningitis was the common clinical manifestation for the 3 horses described in the present report, recurrent fever and bacterial infections of other body systems are important and more common clinical manifestations of immunodeficiency.

Congenital hypogammaglobulinemia or agammaglobulinemia involving 1 or more immunoglobulin classes in horses has been described.20-22 Severe combined immunodeficiency is characterized by agammaglobulinemia and an absence of functional B and T lymphocytes and is transmitted as an autosomal recessive trait in Arabian foals. Another autosomal recessive disease affects Fell pony foals and is characterized by anemia, B-cell lymphopenia with normal T-cell population, and peripheral ganglionopathy. Lack of mature B cells and plasma cells as well as hypogammaglobulinemia are observed in male horses with chromosome X-linked hypogammaglobulinemia. Selective IgM deficiency, in the juvenile or mature (associated with lymphosarcoma) form, is not typically accompanied by B-cell lymphopenia.10 The 3 horses described in the present report had immunologic abnormalities that, in association with signalment and clinical course, could not be categorized as primary congenital immunodeficiency and could not be associated with lymphosarcoma, drug administration, disease, or other reported causes of secondary immunodeficiencies in horses.21
The immunodeficiency in the 3 horses described in the present report can be classified as CVID, a heterogeneous syndrome characterized by hypogammaglobulinemia and recurrent bacterial infections. To our knowledge, CVID involving a horse has been reported only once previously, but other horses with recurrent bacterial infections and atypical immune abnormalities have been described.

In human medicine, CVID affects men and women equally and can develop at any age, although the onset is most frequently during the second or third decade of life. Patients with CVID have a higher incidence of autoimmune disease and malignancy, and a wide range of immunologic abnormalities has been identified in human patients with CVID. Agammaglobulinemia or hypogammaglobulinemia, particularly involving IgM and IgG, is a common feature of this condition. Variable stages of defective B-cell maturation have been identified, including mutations in the heavy-chain gene and defects at the class-switch stage (μ to γ or μ to α) and in the transition from membrane-type IgM to secretion-type IgM. T-cell abnormalities are also common and can manifest as low number of T cells in peripheral blood or as poor activation and proliferation and abnormal lymphokine expression and production. A subgroup of patients with CVID is described in human medicine as having an abnormally low CD4+/CD8+ ratio; the expanded CD8+ population is characterized by activated cytotoxic T cells.

Immunologic abnormalities in all 3 horses described in the present report included decreased responses to mitogens, an abnormal lymphocyte distribution, hypogammaglobulinemia, and failure to respond to immunization. Lymphocyte proliferation assays revealed decreased responses to ConA, PHA, PWM, and LPS in all 3 horses, compared with responses in a control horse. However, interpretation of assay results is influenced by individual physiologic variability in the proliferation response and leukocyte distribution in the isolated cell population. Lymphocytes comprised a smaller percentage of the patient leukocyte population, in comparison to the leukocyte population for the control horse. The high phagocyte-to-lymphocyte ratio in the patient horses was likely attributable to the mild to moderate lymphopenia and chronic, intermittent inflammation. Because CD4+ T cells influence B-cell differentiation through lymphokine production (interleukin-2, interleukin-4, interleukin-5, and γ-interferon) and costimulatory signals, they have been suggested to play a role in defective B-cell maturation and immunoglobulin production in patients with CVID. Absolute T-cell lymphopenia was observed during the acute phase of disease in all 3 horses described in the present report. Therefore, the low number of circulating lymphocytes likely aggravated the impairment of lymphocyte function. Because of the difference in leukocyte distribution, firm conclusions cannot be drawn concerning in vitro lymphocyte responses to mitogens for horses described in the present report. Therefore, even if lack of an appropriate response to PHA and ConA suggests a T-lymphocyte dysfunction, T-cell function in these patients needs further evaluation, including assays that test for cytokine and cell surface molecule expression.

The severity of hypogammaglobulinemia varied among the 3 horses described in the present report, although all 3 had persistent IgM deficiency. Differences in the pattern of hypogammaglobulinemia and changes in immunoglobulin with time are features of CVID in humans. The IgM molecule is expressed on B cells during B cell development and maturation, and the combination of diminished B-cell population and abnormal IgM secretion suggests that B-cell development and maturation may have been affected in all 3 patients. The low or normal concentrations of IgG and IgA could have been sustained by memory B cells or mature B cells stored in secondary lymphoid tissues. Importantly, these patients did not respond with antibody production to tetanus toxoid administration, indicating that in vivo humoral responses were impaired.

A marked decrease in the IgGb isotype concentration was observed in the 2 horses with IgGb deficiency (horses 1 and 2) in the present report. The IgGb isotypes IgGa and IgGb are antibodies that preferentially opsonize and fix complement in horses. A prompt and long-lasting increase in IgGb concentration has been detected after bacterial infection, viral challenge, and allergen inhalation. Suppression of IgGa and IgGb isotype responses to antigen challenge has been detected in horses with immunosuppression resulting from corticosteroid administration. The clinical importance of low concentrations of the IgGb isotype in horses with IgGb deficiency requires further investigation.

The 3 horses described in the present report had adequate phagocytosis, oxidative burst activity, and serum opsonization capacity. The finding of normal in vitro serum opsonization in the presence of hypogammaglobulinemia was unexpected. The innate immune system could potentially compensate for decreased activation of the classic complement pathway in horses with hypogammaglobulinemia through increased activity of the alternative and mannose-binding lectin pathways of the complement system, thereby explaining the near-normal serum opsonization capacity, but further testing is necessary to verify this hypothesis. Beside these considerations, the immune response to live organisms in vivo may be distinct in all 3 and involve more complex aspects in addition to phagocytic function and opsonin concentrations. The recurrence of infection in the absence of antimicrobial treatment would support this hypothesis.

The horses described in the present report were each followed up for > 1 year after the initial clinical signs were observed. The clinical course in these horses was different from that of a previously described horse with CVID, which was euthanatized 3 months after initial signs were seen. In human medicine, the clinical spectrum of CVID is broad, and lower concentrations of IgG, poorer T-cell responses to PHA, and, particularly, lower percentages of B cells in peripheral blood are associated with a higher risk of death. In horses, it appears that CVID may also be associated with different degrees of severity.

The management of human patients with CVID involves prompt treatment of all infections and complications. Immunoglobulin replacement therapy is
also used but would be cost-prohibitive in horses. Other treatments, including long-term, low-dosage administration of interleukin-2, are being studied, but there is as yet insufficient evidence to recommend their use. Long-term antimicrobial treatment, as reported for horses 1 and 2, may be beneficial in some patients but raises concerns about antimicrobial resistance and opportunistic infections. Although treatment recommendations are difficult to formulate, general recommendations may be considered. For instance, on 2 occasions, horse 2 developed severe systemic disease consequent to a change of environment. Thus, it is reasonable to think that reducing the exposure to risk situations (eg, horse shows, prolonged transportation, and changes of environment) may decrease the likelihood of infection.

The low number of horses in which CVID had been diagnosed along with the poor definition of the disease in humans and horses makes comparison of CVID between species difficult. Similarly to the condition described in humans, CVID appears to be characterized by a late onset in horses and affects various breeds and both sexes. In both species, CVID is characterized by variable patterns of hypogammaglobulinemia or agammaglobulinemia and a lack of response to vaccination. Respiratory tract infections represent the most common presentation of CVID in humans, followed by meningoencephalitis, gastrointestinal tract disease, and autoimmune and lymphoproliferative disorders. Respiratory tract disease was documented in a previous horse with CVID, but none of the horses in the present report had clinical signs of respiratory tract disease. The severity of B-cell lymphopenia observed in horses 1 and 2 and in a previous horse is not typical of CVID in humans and, when present, is associated with a higher risk of death. Finally, horses in the present report were successfully managed without immunoglobulin replacement therapy. Although the reasons why horses survived without immunoglobulin replacement therapy remain to be elucidated, the adequate phagocytosis, oxidative burst activity, and serum opsonization capacity may have played a role. The high rate of bacterial meningoencephalitis in human patients with immunodeficiency and the finding of CVID in 3 adult horses with bacterial meningitis suggest that immune function testing may be warranted in adult horses with suppurative meningitis.

References

34. Weldon AD, Zhang C, Antczak DF, et al. Selective IgM defi-