

Potomac Horse Fever

INTRODUCTION

Potomac horse fever is an acute enterocolitis of equines caused by a group of closely related intracellular-occurring ehrlichias collectively known as *Neorickettsia risticii*, first recognized in 1979 along the Potomac River in Maryland, USA.[29] *Neorickettsia risticii* infection in horses has been referred to as Potomac horse fever, equine monocytic ehrlichiosis, and equine ehrlichial colitis. It has been recognized to occur throughout North America. The original term, "Potomac horse fever", was coined by a television reporter covering the original epidemic along the Potomac River. Although it is the least descriptive term, its wide acceptance and its high level of recognition ensures that it will remain the most common term for the disease. Equine ehrlichial colitis is a more descriptive term for the original syndrome of fever, anorexia, depression and diarrhea. Even mild cases not manifesting diarrhea have evidence of colitis. Equine ehrlichial abortion is an appropriate term for the abortion syndrome caused by *N. risticii*.

ETIOLOGY

The causative organism of Potomac horse fever is a member of the tribe Ehrlichieae.[23] Based on nucleotide sequence, the genus *Ehrlichia* is phylogenetically incoherent. *Neorickettsia risticii* is most closely related to *Neorickettsia sennetsu* and *Neorickettsia helminthoeca* (levels of sequence similarity, > 95%).[55] Although only definitively proven for *N. helminthoeca*, it is likely that these three organisms also share the unique property of being the only known obligate intracellular bacteria that are transmitted via a helminth vector.

Although *N. risticii* grows readily in a number of tissue culture lines as long as antimicrobials are not used in the culture media, the continuous murine macrophage cell line P388D1 is frequently used for isolation and propagation. The original Maryland isolate grows readily in this cell line initially appearing as a cluster of singly occurring elementary bodies followed by the development of initial bodies and later forming morulae (mature inclusions). Heavily infected cells eventually disintegrate, releasing loosely packed groups of organisms held together by cytoplasmic stroma. [69]

There is considerable biological diversity of *N. risticii* isolates from clinical cases of Potomac horse fever. Morphologically, some form large cytoplasmic morulae (inclusions) whereas others form small morulae or are individually dispersed in the cytoplasm in murine P388D1 cells.[11] Similarly, patterns of antigenic proteins may differ considerably between isolates.[11] The sequences of the 16S rRNA genes of isolates may differ between each other more than from the next most closely related *Neorickettsia* sp., *Neorickettsia sennetsu*. It is evident that Potomac horse fever is not caused by one

Neorickettsia but rather several closely related but distinct *Neorickettsia* spp.[83] This antigenic variation of isolates causing the same clinical disease has resulted in incomplete protection from the first generation of vaccines which all originated from the same type strain of *N. risticii*.

EPIDEMIOLOGY

Potomac horse fever is confined to North America where it has been reported in a wide variety of geographic regions in most US states and Canada. Surveys in the USA have shown that 16 to 33% of horses with no history of illness have antibodies to *N. risticii*[18, 39, 65] and that these are seasonal fluctuations with the highest number of seropositive horses and highest clinical occurrence in July, August and September.[18, 39] Over a five year period 70% of 900 clinical cases recorded in Maryland occurred during the same months.[53]

Clinical disease shows an unusual sporadic pattern with a low prevalence rate (< 5%) on any one farm despite its frequent occurrence in an endemic area. In fact, when multiple cases occur on a large farm, the pattern is also temporally (within the season of the disease) and geographically (among the pastures and barns on the farm) sporadic. Occasionally an epidemic form occurs which is characterized by a high attack rate (20 to 50%) on an individual farm. The disease may be concentrated on a particular farm or race track, resulting in an outbreak of a large number of horses simultaneously. The reason for this epidemiological variation is unknown.[45, 48]

The mode of transmission has been extensively studied but significant questions continue to exist. Direct contact transmission does not occur.[44] The seasonal occurrence lead to early investigation of a possible arthropod vector. Common vectors of other ehrlichial agents are ticks.[67] The only adult tick found feeding on horses along the Potomac River during the original epidemic in the early 1980s was *Dermacentor variabilis*.[9, 72] The larval and nymphal stages of this tick feed on small ground mammals such as the white-footed mouse (*Peromyscus leucopus*).[9] Mice are highly susceptible to experimental *N. risticii* infection.[24] However, attempts to transmit *N. risticii* to horses using field captured adult ticks (*Dermacentor variabilis*) from endemic farms failed.[72] Furthermore, larval and nymphal stages of *Dermacentor variabilis* fed on ehrlichiaemic mice did not transmit the organism to other mice or to ponies[20, 30] and white-footed mice infested with immature *Dermacentor variabilis* and living in endemic areas of the disease have consistently been found to be seronegative.[9] Dogs, the primary host of adult *Dermacentor variabilis*, are uniformly seronegative on endemic farms[53] despite being susceptible to infection.[68] *Dermacentor variabilis* is not the vector of *N. risticii* and attempts to transmit the pathogen with other ticks including *Rhipicephalus sanguineus*, *Ixodes scapularis*, and *Amblyomma americanum* have also failed.[20]

Despite the wide experimental host susceptibility of *N. risticii*,[13, 24, 68, 77] non-equine mammals in endemic areas show a surprisingly low frequency of seroconversion.[9, 19,

53, 71] One investigation revealed that a high percentage of cats on some endemic farms were seropositive,[53] but transmission studies using the cat flea *Ctenocephalides felis* and the chigger mite *Neotrombicula whartoni*, both common ectoparasites of cats on endemic farms, failed when *N. risticii* infected horses or mice were used as donors and mice were used as recipients.[71]

The ehrlichiaemia that occurs during the acute stage of the disease makes transmission by haematophagous flies possible. The role of adult stable flies (*Stomoxys calcitrans*) has been investigated:[8] despite demonstrating ehrlichiaemia in donor horses, recipient horses did not seroconvert or develop signs of Potomac horse fever and recovery of *N. risticii* from the flies was not successful.

All evidence indicate that blood sucking arthropods are not responsible for transmitting *N. risticii*. Oral infection produces clinical disease which is identical to that seen when the organism is given intravenously.[44] If horses are exposed orally to a sufficient number of viable *Neorickettsia*, infection will occur. Large numbers of *N. risticii* are shed into the lumen of the colon in exfoliating epithelial cells.[64] Thus large numbers of *Ehrlichia* are present in the feces of infected horses.[6, 38] However, direct contact do not result in transmission,[44] so it seems unlikely that contaminated feces plays a direct role in the transmission of the disease. Indirect oral transmission may occur through concentration of the *Ehrlichia* in a vector. *Neorickettsia helminthoeca* and *N. elokominica*, two other members of the tribe *Ehrlichieae* which share common antigens[59] and DNA homology[55] with *N. risticii*, are transmitted through ingestion of infected helminths.[22, 23] The metacercaria of the salmon fluke (*Nanophyetus salmincola*) carries these pathogens. Dogs are infected by ingestion of fluke infested salmon or trout. Similarly, *N. risticii* may be concentrated in infected helminths carried by arthropods which are inadvertently ingested. *Neorickettsia sennetsu*, a closely related pathogen of humans, has been suspected of being transmitted by ingestion of a fish nematode.[28] The involvement of an arthropod vector or helminth would be consistent with the seasonality of the disease.

Recently a reservoir of *N. risticii* has been identified in trematode cercariae (virgulate cercariae) parasitizing freshwater snails and aquatic insects in two geographic distinct areas of the USA.[2, 10, 25, 56, 58] The trematodes which can become infected with *N. risticii* appear to have a broad intermediate host range, having been found in abundance in adult and immature forms of the following aquatic insects: caddisflies (*Trichoptera*), mayflies (*Ephemeroptera*), damselflies (*Odonata, Zygoptera*), dragonflies (*Odonata, Anisoptera*), and stoneflies (*Plecoptera*).[10] In one study, the prevalence of *N. risticii* was 32% in 13 of 17 aquatic species of insects acting as hosts for the trematodes.[10] There is also evidence that bats help maintain the natural reservoir.

The discovery of an aquatic reservoir is not surprising considering the early observations that the disease outbreaks centered along major rivers.[48] This finding could easily explain the observation that the disease appears to be associated with areas and not horses in the areas. One of the original index farms along the Potomac River was abandoned and

left without horses for several years because of high prevalence of the disease. It was then repopulated with a new herd. Potomac horse fever reemerged on the farm during the first spring thereafter, a high attack rate being recorded in the newly introduced horses.

The connection between the aquatic reservoir and transmission to horses has not been clearly characterized. Transmission attempts using naturally infected trematodes through skin contact with water harbouring *N. risticii*-infected cercariae, through ingestion of water harbouring *N. risticii*-infected cercariae or through ingestion of different aquatic insects (caddisfly larvae [Limnephilidae], adult caddisflies [Leptoceridae, Limnephilidae], nymph and adult mayflies [Heptageniidea] and stonefly nymphs [Perlodidae]) harbouring *N. risticii*-infected metacercariae were only successful in one horse which received large numbers of adult caddisflies. Two other horses receiving fewer adult caddisflies did not develop disease, seroconvert, develop ehrlichemia or shed *Neorickettsia* in their feces.[33] Considering the susceptibility of horses to oral infections, it is surprising that it was not easier to transmit the organism through ingestion of the trematodes.[44] The failure to do so is probably associated with lack of sufficient viable *Neorickettsia*.

How horses would ingest sufficient numbers of these aquatic insects to result in infection has not been explored. Caddisflies spend their life primarily on the water or in vegetation near water. They may be attracted by lights at night. When the ecosystem of horses and caddisflies, or other aquatic insects, would allow for transmission is unclear. Another issue that needs resolution is the observation that cases of Potomac horse fever can occur significant distances from major waterways as well as in horses that have no apparent access to streams or other aquatic environments.[42, 45, 48]

The fecal shedding of *N. risticii*, which appears to be a consistent finding in infected horses,[6] is an ideal way to seed aquatic environments with the organism, and introduction of recently infected horses could conceivably convert a previously disease-free area into one potentially endemic for the disease, if aquatic environments do prove to harbor the source of endemic disease in horses. It is apparent that, although a freshwater reservoir is probably important in the epidemiology of *Neorickettsia risticii*, many questions have been left unanswered.

PATHOGENESIS

Although the mode of transmission is unknown, it is clear that within hours of infection the *N. risticii* can be found in a subpopulation of blood monocytes. Although the pathogen is readily phagocytized by monocytes,[84] it appears to elude the host's defense mechanisms by inhibiting lysosomal fusion with phagosomes.[82] The ehrlichemia persists throughout and beyond the clinical period. The pathogen has a predilection for mucosa of the cecum and large colon but is occasionally found in jejunum and small colon mucosa.[64] Intestinal epithelial cells, mast cells and macrophages are the targets of infection. Lesions are confined to the intestinal tract.[12] The inflammatory response to *N. risticii* infection, which appears to vary in intensity, is mediated through cytokines.

Preliminary studies in mice suggest that increased macrophage production of interleukin-1 alpha, but not tumour necrosis factor-alpha, interleukin-6 or prostaglandin E2, may be primarily involved in the pathogenesis of the disease.[79]

Neorickettsia risticii causes significant immune depression in mice and measurable alterations of the equine immune system, probably through modification by inflammatory cytokines. Whether or not the immune depression is clinically important in horses is unclear. Infected mice show evidence of significant depression of both humoral and cellular immunity[62] and develop lymphoid depletion in lymphoid tissues.[63] Although infected horses have a transient decrease in antibody production[16] and lymphocyte function,[62] no histologic lesions of the lymphatic system develop.[12, 63] In rickettsial infections antibodies can be protective when they block the pathogen's attachment to or penetration of host cells.[21] Anti-*N. risticii* equine IgG inhibits *N. risticii* internalization and interferes with the metabolic activity of *N. risticii*, rendering them incapable of proliferation in host cells and resulting in the eventual destruction of the organisms.[36] Opsonization of *N. risticii* with anti-*N. risticii* serum renders *N. risticii* more susceptible to macrophage destruction.[84] Mice can be passively protected from *N. risticii* infection by antibody transfer.[26] However, *Neorickettsia* can be isolated from animals in the presence of a rising antibody titer,[14] and presence of antibodies does not always correlate with clearance of *Neorickettsia* or with presence of protective immunity.[60] Effective neutralizing antibodies are the important subset of the general antibody response to ehrlichial infections. In horses, clinical disease occurs in the face of rising antibody titers. Modulation of the immune response by the pathogen so that the initial antibodies are not protective may be an important aspect of the pathogen's virulence.

CLINICAL SIGNS

There is considerable variation in clinical manifestations of Potomac horse fever. The clinical signs include fever, depression, anorexia, ileus, colic, diarrhea and laminitis.[40, 45, 48, 51, 52, 69] It is a common misconception that typical cases show most of these signs. On the contrary, any combination of these signs may be present. Common to all cases are the clinical manifestations of colitis which does not always result in colic or diarrhea. Mild colitis can result in depression and anorexia, with or without fever. Normally formed feces may be passed without signs of colic being present. Cases with equivocal signs of gastrointestinal tract involvement typically have, on careful auscultation of the abdomen, a remarkable decrease in borborygmal sounds, confirming gastrointestinal disease and long periods of silence broken by short bursts of loud high pitched sounds produced by gas passing through a tense intestine with a gas-fluid interface will be revealed. Simultaneous auscultation and finger percussion often results in a high pitched resonance in the dorsal abdomen. Simultaneous auscultation and ballottement of the abdomen reveal splashing sounds confirming a gas-fluid interface. These signs of ileus are the most consistent clinical findings in all cases, and take on added significance when they occur in the face of an absence of clinical signs of colic, and the passage of normally formed feces.

The fever is frequently biphasic, although the first phase is often not detected. The initial increased rectal temperature ranges from 103 to 106°F (39.4 to 41.1°C) but, since it is not accompanied by other signs, the presence of this initial fever will not be realized unless the horse's temperature is measured frequently. It typically resolves within hours and is followed in 3 to 7 days by a more persistent fever accompanied by other clinical signs.

Although diarrhea is often thought of as a cardinal sign, it develops in less than 60% of cases.[40] When present, fecal consistency can vary from cow-like to watery, pipe-stream, but the diarrhea is not as profuse as that associated with enteric salmonellosis in horses. The duration of diarrhea may be as short as one day and rarely lasts as long as 10 days. Chronic diarrhea is not a manifestation of *N. risticii* infection.

Decreased feed intake may be a prominent sign. Some horses may have complete anorexia for up to 10 days in the absence of other signs. The major manifestation in other cases may be a profound ileus evident by severe colic, severe toxemia and dehydration. In some outbreaks severe laminitis in all four hooves may occur in up to 40% of affected horses, but in most areas it is a rare sign.[40, 45, 48] The laminitis, when present, may be the major manifestation of disease and result in humane euthanasia of the horse within days of onset. More often, however, it occurs along with other signs, is mild and completely resolves within 3 to 5 days. A few cases develop chronic laminitis.

The course of disease without therapeutic intervention is usually 5 to 10 days. Chronic manifestations, with the exception of laminitis, do not occur. The case fatality rate of untreated clinical cases varies from 5 to 30%. Some fatalities are related to an apparent uncontrolled sepsis, the horse suddenly dying despite not being severely dehydrated or having extreme acid/base or electrolyte imbalances. This may be an extreme inflammatory response orchestrated by a modified systemic inflammatory response syndrome response. Other horses may have to be euthanized because of severe laminitis.[40]

Neorickettsia risticii can cause abortion. Experimentally, naive pregnant mares infected between 100 and 160 days of gestation abort between 190 and 250 days of gestation. The mares developed typical signs of Potomac horse fever and appear to have recovered fully before aborting. Abortion is accompanied by histological placentitis and a retained placenta in most cases. Foals which are carried to term may or may not have a presuck titer of serum antibodies to *N. risticii*. In either case full-term foals are healthy and are not ehrlichemic.[15, 31, 32]

PATHOLOGY

The leukocyte count during the early phases of clinical signs may be variable. Leukopenia with a neutropenia and lymphopenia producing a hemogram similar to that typically seen in equine salmonellosis may be present, but many horses have normal leukocyte counts despite other signs of enteritis. A marked leukocytosis can occur within a few days of onset of clinical signs in some cases. A total white blood cell count of 20,000 to 30,000

cells/ μ l may be found after an initial leukopenia. Although this profound leukocytosis does not occur consistently with *N. risticii* infection, it is a rare finding in mature horses suffering from most other diseases. If it is detected in a horse with consistent clinical signs, suspicion of Potomac horse fever should be high.

Gross necropsy, even on fatal cases of Potomac horse fever, is generally unimpressive. The most consistent post-mortem findings are increased amounts of fluid contents in the cecum and large colon, and focal areas of mild hyperemia primarily in the cecum and large intestine mucosa but occasionally also of the small intestine. The intestinal wall is not edematous and the mucosa is intact.[12] A histologic colitis is usually evident and *Neorickettsia* can be identified with a silver stain[76] or electron microscopy and detected by tissue PCR.[41] The histologic appearance is somewhat variable but generally includes multifocal necrosis limited to the mucosa with a mixed inflammatory infiltrate. The histologic lesion often appears mild in relation to the severe clinical disease which leads to a fatal outcome.

In addition to the placentitis, histologic examination of fetal tissues show a remarkable colitis, periportal hepatitis and lymphoid hyperplasia of the mesenteric lymph nodes and spleen.

DIAGNOSIS

Although the epidemiology, clinical presentation, hematology findings and response to treatment may all be suggestive of Potomac horse fever, its diagnosis is based on serology, PCR and/or isolation of *N. risticii* in cultures. Although the organism does parasitize circulating monocytes and can be seen with common hematology stains, since only a small percentage of circulating monocytes are infected, examination of peripheral smears is not a rewarding diagnostic procedure.

Neorickettsia risticii can be isolated from monocyte cultures of blood within days of experimental infection and consistently until 30 days after infection.[44] *Neorickettsia risticii* may also be cultured from affected fetal tissues but this may be difficult because of bacterial contamination. The placenta is usually retained, especially when the period between infection and abortion is long. However, isolation and growth of *Neorickettsia* in antibiotic free tissue culture (co-cultivation of tissue or blood monocytes with murine P388D1 cells) are research techniques not easily adapted to diagnostic laboratories. Although a definitive diagnosis of Potomac horse fever requires that *N. risticii* be isolated, in practice reliance is placed on serology or PCR for its diagnosis. Serology is not a straight forward diagnostic technique because of the somewhat unique kinetics of antibody production stimulated by *N. risticii*. Infected horses have a rapid rise in fluorescent antibody titer which usually begins before onset of clinical signs. Antibody levels peak quickly, reaching a high level within a few days. In the absence of a blood sample for serology taken in the early stages of the disease, detecting a significant rise in antibody titer may be impossible. Even samples taken within hours of the onset of clinical signs can show fluorescent antibody titers of 1:2560 or higher. As a result, a decrease in titer is as common as a rise in titer and must be taken into account when antibody titers in

acute and convalescent serum samples are compared. Thus a four fold or greater increase or decrease in florescent antibody titer can be considered diagnostic. Approximately half of acutely affected clinical cases will have no significant change in titer because the antibody level has reached a plateau before the first sample is drawn.[35]

The determination of antibody titers in single serum samples serves no purpose. Although clinical cases will have titers greater than 1:80, there is no correlation between height of titer and likelihood of acute disease. Antibody titers in horses that have been exposed to Potomac horse fever for longer than a year before antibody determination are done and may have titers as high as 1:5120. On the other hand, if a horse is found to have a negative titer after clinical signs develop, it is probable that it is not suffering from the disease and other diagnoses should be pursued. Treatment with oxytetracycline early in the clinical course of the disease does not affect the development of high antibody levels. Horses which have been vaccinated with a Potomac horse fever vaccine may have titers as high as 1:640. In general, titers consistently decrease after vaccination and after six to nine months have disappeared. When vaccinated animals develop the clinical disease (e.g. when a vaccine “break” occurs) antibody titers may rise dramatically. In such cases antibody titers are often over 1:100,000 despite the presence of clinical signs of the disease.[35, 40, 70]

The first blood sample for diagnostic serum antibody determinations should be taken early in the course of the clinical disease and the second five to seven days after the first to maximize the chance of seeing a significant change. This short time interval between the first and second antibody determinations is contrary to what is generally the case for most other infectious diseases in which, for diagnostic purposes, reliance is also placed on the differences in antibody levels in acute and convalescent phase sera, the latter being determined two to three weeks or longer after the former. In Potomac horse fever this longer time interval has no advantage. It is of utmost importance to have the antibody levels in both the acute and chronic phase sera determined simultaneously in the same laboratory.

Great care must be taken in choosing a laboratory to assay serum samples. The most common technique used is an indirect fluorescent antibody test. Laboratories adept at running other indirect fluorescent antibody assays may still have difficulty with the procedure for Potomac horse fever. Inconsistent antigen preparations, improper use of positive and negative controls, confusing nonspecific fluorescence, and inexperience with identifying specific fluorescence can all lead to erroneous results. The most consistent results will be obtained from laboratories which frequently run the assay and take great care in running both negative and positive controls.

A recent study has shown that even when performed by experienced research laboratories, the serologic test results may not be accurate. Serum samples from suspect cases and a sample from an experimentally infected horse were sent blindly to several university and private laboratories. Most laboratories reported high titers in many of the samples. Close scrutiny of the samples by Western blot analysis showed that the only true positive

sample came from the experimentally infected horse. The rest were false positives.[34] This suggests that caution should be used in interpreting the results of serological tests when used for diagnostic purposes. Serology should be abandoned in favor of more accurate diagnostic methods, as they become available.

A polymerase chain reaction (PCR) technique has been adapted to detect *N. risticii*. [3, 5, 6, 38, 42, 57] *Neorickettsia* can be identified in blood samples and tissues [41] without the need for culturing, and it takes less than a day. Although false negative and false positive results [54] can occur, with proper controls the PCR assay should be a rapid, definitive diagnostic test and should replace serology as the preferred clinical diagnostic method. This testing procedure should not be viewed as a “gold” standard since doing so may lead to unnecessary morbidity and mortality from unnecessary antimicrobial therapy resulting in antimicrobial induced complications (antimicrobial associated enteritis, renal disease, etc.) as has recently been reported in human medicine associated with the diagnosis of Lyme borreliosis. [37, 50]

Ehrlichial abortion can be tentatively diagnosed by histologic examination of fetal colon. In fact, the colonic lesions are as helpful in establishing a diagnosis of *N. risticii* abortion as the fetal liver lesions are in equine herpesvirus abortion. Confirmation is possible through a PCR assay of fetal tissues. [41]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of Potomac horse fever includes salmonellosis, intestinal clostridiosis, NSAID toxicity, antimicrobial associated enteritis, colitis X, fungal colitis and toxic enteritis.

CONTROL

Neorickettsia risticii is sensitive *in vitro* to demeclocycline, doxycycline, oxytetracycline, and minocycline. [61] Oxytetracycline is the drug of choice for treating *N. risticii* infection in horses. The organism is an obligate intracellular parasite that survives within phagosomes in macrophages by inhibiting phagosome-lysosome fusion. [82] This inhibition is reversed *in vitro* by oxytetracycline, resulting in destruction of the *Neorickettsia* by the host cell. [82] Oxytetracycline may interfere with production of a cell wall protein that is responsible for the phagosome-lysosome fusion inhibition.

Oxytetracycline administered intravenously at the dosage rate of 6.6 mg/kg once a day for 5 days is a very effective treatment for Potomac horse fever when given early in the course of the disease. A response to treatment is seen within 12 hours as noted by a decrease in rectal temperature, followed by an improvement in appetite, attitude and borborygmal sounds. [47] Diarrhea, if present, does not respond to oxytetracycline therapy. [47] Since destroying the pathogen would be expected to take longer than 12 hours, the rapid clinical response to treatment implies that metabolic by-products, acting through the production of cytokines resulting in the systemic inflammatory response

syndrome, may be responsible for many of the clinical signs. Once treatment is begun, progression of the disease stops. The response to oxytetracycline therapy is so dramatic, that it can be used to strengthen a clinical diagnosis. If the diagnosis is correct, a response to therapy should be clearly seen within 12 hours (with the exception of a change in diarrhea). If no improvement occurs within 24 hours, the diagnosis should be reconsidered. If therapy is begun early in the course of the clinical disease, clinical signs resolve by the third day of treatment and no more than five days of therapy are needed.[47] If a relapse occurs after discontinuing therapy, a second course of oxytetracycline is usually as successful as the first.

When treatment is begun early in the course of some rickettsial diseases, relapses may occur.[27, 75, 78] Relapses are thought to be due to lack of sufficient time for adequate immune stimulation of the host resulting in a lack of protection.[75] In experimental *N. risticii* infection, relapses do not occur when therapy is initiated early in the clinical course.[47] However, treatment during the incubation period merely prolongs the incubation period but does not prevent clinical disease from developing.[49] Early treatment does not interfere with the serologic response or the development of protection.[47]

A combination of erythromycin (25 mg/kg) and rifampin (10 mg/kg) administered orally twice a day is also effective in treating Potomac horse fever when given early in the course of clinical disease.[43] The response to this treatment is similar to that to oxytetracycline, with the exception that there is a slightly longer delay before the animals becomes afebrile (24 hours vs.12 hours).[43, 47] Such a delay in the induction of apyrexia has also been observed when rifampin has been used to treat rickettsial infections in humans. The time period between initiation of therapy and return to normal of other physical findings is similar for both treatment regimes.[43, 47] Although no experimental evidence is available in horses, it is considered that doxycycline administered orally should also be effective in the therapy of the disease.[62]

As in any horse suffering from colitis, the administration of fluids is an important consideration. Fluid and electrolyte replacement therapy should be tailored to suit the individual case. Many of the clinical signs in acute colitis cases can be attributed to inflammatory cytokine responses. These cases may benefit from therapy with anti-inflammatory mediators. When choosing such therapy, it should be borne in mind that experimental evidence suggests interleukin-1 alpha may be much more important than tumor necrosis factor alpha, interleukin-6 or prostaglandins in the inflammatory response caused by *N. risticii*. [79] Secondary salmonellosis is not uncommon in horses suffering from Potomac horse fever and this possibility should not be overlooked. For this reason, and because a definitive diagnosis is often difficult, it is felt that horses suspected of having Potomac horse fever should be isolated despite the lack of direct contact transmission.

Until the mode of transmission is clarified and the vector unequivocally identified, attempts to prevent exposure are unlikely to succeed. Although aquatic environments

appear to be a location of one reservoir for the organism, there is no current evidence that the avoidance of such areas will prevent infection since the disease also occurs in horses which have had no direct contact with them.

After infection with *N. risticii*, protective immunity develops to the infecting strain as evidenced by clinical protection to reinfection for as long as 20 months.[46] Protective immunity can be passively transferred,[26] suggesting that it is primarily humoral. Neutralizing antibodies to specific protective antigens appear to be an important aspect in the induction of an effective immunity.[17, 66, 74, 81] These antibodies appear both to block internalization of *N. risticii* and to interfere with its metabolic activity in host cells, rendering it incapable of intracellular proliferation, and result in its eventual destruction.[36] Thus vaccine induced immunity should be possible.

A number of inactivated, partially purified, whole cell vaccines are commercially available in the USA. Vaccination has been reported to protect 78% of experimentally infected horses from all clinical manifestations of Potomac horse fever except, in most cases, fever (22% had no clinical signs, 56% developed fever only, 22% developed fever and other signs of disease).[70] Vaccination-induced protection is incomplete at best, which may be associated with poor stimulation of neutralizing antibodies by the vaccine.[17] The protection induced by vaccination wanes much more rapidly than that induced by experimental infection. Only 50% of vaccinates are fully protected at six months after vaccination,[73] and protection decreases to 33% after nine months.[4] Based on this evidence, revaccination at 4 month intervals appears to be necessary to maintain a reasonable likelihood of protection. In most endemic areas the disease occurs seasonally. In these areas, if any of the currently available inactivated vaccines are used, horses should be vaccinated in the spring, one month before the first cases are expected and again four months later, if cases are still occurring in the area.

During the past few years, protection by vaccination seems to have become less efficacious. Initially, after the introduction of the vaccines, vaccinated animals which did develop disease appeared to have a mild form. More recently, however, some vaccinated horses have developed the severe natural disease and some of which died despite the fact that they were apparently adequately vaccinated. In some areas, the prevalence rate of the disease and treatment expenses are not decreased by vaccination.[1] The reason for these vaccine failures may be associated with inconsistent antigen preparation or, more likely, it is associated with the appearance of several genetically distinct strains of *N. risticii* which vary in their protective antigens.[7, 17, 80] All vaccines currently available contain only the strain that was originally isolated. Polyvalent recombinant vaccines using identified protective antigens may, in future, become available which should increase the efficacy of protection afforded by vaccination.