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Pathological evidence of ehrlichiosis among calves inoculated with *Ehrlichia chaffeensis*

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Abstract

An immunocompetent animal disease model based on infection with *Ehrlichia chaffeensis* would facilitate research toward understanding mechanisms responsible for the broad range of clinical signs associated with human monocytic ehrlichiosis (HME). Adaptability to experimental feeding of various tick species and stages and to testing therapies comparable to those for human diseases are additional advantages of large animal models. Herein we summarize pathology reports for calves that developed fatal disease after experimental inoculation with *E. chaffeensis*. Elevated liver enzyme levels and lung pathology among these deceased calves corroborated earlier reports of severe HME. Thus, an experimental disease model based on infection of outbred immunocompetent hosts with *E. chaffeensis* could be within our grasp for the first time.

Keywords

Ehrlichia chaffeensis; human monocytic ehrlichiosis; large animal disease model

INTRODUCTION

Human monocytic ehrlichiosis (HME) has a clinical spectrum that spans from asymptomatic to severe manifestations in multiple organ systems and death (as reviewed by Paddock and Childs).[1] Our understanding of HME is delayed in part by the absence of a reliable, reproducible animal disease model that is based on infection with *Ehrlichia chaffeensis*, the etiologic agent of HME. Although several mammals are susceptible to infection with *E. chaffeensis* under natural or experimental conditions, to date only naturally infected primates and experimentally infected immunodeficient mice were shown to develop clinical disease.[1, 2] Thus, the most promising immunocompetent disease models to date include infection of mice or dogs with several *Ehrlichia* spp. other than *E. chaffeensis*.[1, 3, 4] We recently reported that dairy calves were susceptible to experimental infection with *E. chaffeensis*.[5] Infections in calves were confirmed by detection of *E. chaffeensis* in peripheral blood and xenodiagnosis by ticks that acquired the pathogen as nymphs and were PCR-positive after molting to the adult stage. The purpose of this report is to summarize pathology reports for three calves from the aforementioned study, each of which died after experimental inoculation with one of three different *E. chaffeensis* strains.

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METHODS

Holstein bull calves (2 months old) were purchased from a local dairy and observed under quarantine conditions for two weeks prior to intravenous inoculation with Arkansas, St. Vincent or 91HE17 strains of *E. chaffeensis* (two calves per strain) in 1.6×10^7 infected DH82 cells. Two calves were inoculated with uninfected DH82 cells as controls. Among these calves, A33, A36 and A38, which were inoculated with Arkansas, St. Vincent and 91HE17 strains, respectively, became lethargic and developed progressive muscular weakness and eventually died 6–13 days post-inoculation (dpi). The calves that expired were submitted for necropsy to the Ohio State University College of Veterinary Medicine.

RESULTS AND DISCUSSION

The necropsy reports for calves A33, A36 and A38 are summarized in Table 1. Analyses of A33 and A36 sera prior to their deaths demonstrated markedly elevated aspartate aminotransferase and creatine kinase levels, with concurrently low levels in phosphorus and potassium. Calcium, total protein, and urea nitrogen were all within normal ranges for both calves. At necropsy serous fat atrophy was observed in all three calves. Hepatic congestion was observed in calf A36.

Elevated liver enzyme levels in peripheral blood, commonly associated with severe HME,[1, 6, 7] were obvious in both calves tested. Interestingly, *E. chaffeensis*-infected lemurs also developed elevated serum aminotransferase levels similar to human patients.[2] Lowered serum potassium and phosphorus levels in calves A36 and A33, and elevated leukocytes and protein levels in the CSF of A33 and A38, were also reported in cases of severe HME.[8] Although elevated creatine kinase levels can indicate recumbency in larger cattle and horses, elevated creatine phosphokinase levels were associated among some cases of HME with multiple system involvement.[1, 9]

Lung tissue sections from A33 showed acute, diffuse, moderate pulmonary congestion. Edema was noted from the cut surface of the cranioventral lobes and hilar region of the lungs, which were firm and darker than normal. Pulmonary edema was noted upon examination of cut lung parenchyma of A36. Atelectasis of small foci of the right cranial lung lobe was also noted. For A38, lung tissue sections revealed acute, marked and diffuse pyogranulomatous bronchopneumonia with multinucleated giant cells.

Frequent and sometimes severe respiratory system involvement is another seemingly consistent clinical finding among HME patients.[8] Similarly, lung lesions observed at gross necropsy of calves A33, A36 and A38, were consistent with this clinical feature of HME. Although lung histopathology of calf A38 was consistent with a report of similar findings in a human patient,[8] the bronchopneumonia may have also reflected a pre-existing condition.

Cerebrospinal fluid (CSF) samples were collected from A33 and A38 at necropsy. Elevated total protein (94 mg/dl), an elevated leukocyte count (50 cells/μl, comprised of lymphocytes (53%) and monocytes (47%)), and moderate numbers of erythrocytes (333 cells/μl) were noted upon analysis of CSF from A33. Erythrophagocytosis (a sign of ehrlichiosis) was evident and inclusions were observed within leukocytes, but these inclusions could not be conclusively identified as morulae. For A38, CSF cytology showed a prominently elevated protein level (340 mg/dl), and the total leukocyte count was elevated at 41 cells/μl. Microscopic examination of brain sections, including the cerebellum and brainstem, did not reveal significant lesions in any of the calves. However, CSF analyses supported diagnosis of meningitis, even in the absence of histological lesions, because CSF cytology is a more sensitive indicator of inflammation than histopathology.[10]

Clinical signs among these calves appeared after 3–4 weeks of observation, which included the 6–13 dpi. Another calf, A37, suffered the same crisis as the deceased calves at 16 dpi but survived due to extensive supportive therapy. This calf then displayed signs consistent with mild ehrlichiosis, became PCR- and IFA-positive, and served as a source of tick infection.[5] Importantly, no signs of infection or disease were observed for the two negative controls that were each inoculated with non-infected DH82 cells, and all calves used for this experiment were exposed to the same conditions and DH82 host cell stock.

In conclusion, this appears to the first report of host pathology resembling severe ehrlichiosis in immunocompetent hosts experimentally infected with *E. chaffeensis*. Elevated liver enzyme levels and lung pathology among these fatalities corroborated earlier reports of severe HME. Thus, an experimental disease model based on infection of outbred immunocompetent hosts with *E. chaffeensis* could be within our grasp for the first time. Further work is needed to optimize this model and to determine if it would be useful to evaluate factors responsible for exacerbation of HME, as well as prophylactic and therapeutic approaches to alleviation of life-threatening forms of this disease.

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REFERENCES

1. Paddock CD, Childs JE. *Ehrlichia chaffeensis*: a Prototypical Emerging Pathogen. Clin. Microbiol. Rev 2003;16:37–64. [PubMed: 12525424]
2. Williams CV, Van Steenhouse JL, Bradley JM, Hancock SI, Hegarty BC, Breitschwerdt EB. Naturally occurring *Ehrlichia chaffeensis* infection in two prosimian primate species: ring-tailed lemurs (*Lemur catta*) and ruffed lemurs (*Varecia variegata*). Emerg. Infect. Dis 2002;8:1497–1500. [PubMed: 12498671]
3. Feng HM, Walker DH. Mechanisms of immunity to *Ehrlichia muris*: a model of monocytotropic ehrlichiosis. Infect. Immun 2004;72:966–971. [PubMed: 14742542]
4. Stich RW, Schaefer JJ, Bremer WG, Needham GR, Jittapalapong S. Host surveys, ixodid tick biology and transmission scenarios as related to the tick-borne pathogen, *Ehrlichia canis*. Vet. Parasitol. 2007 Manuscript accepted.
5. delos Santos JRC, Boughan K, Bremer WG, Rizzo B, Schaefer JJ, Rikihisa Y, Needham GR, Capitini LA, Anderson DE, Oglesbee M, Ewing SA, Stich RW. Experimental infection of dairy calves with *Ehrlichia chaffeensis*. J Med Microbiol. 2007 Manuscript accepted.
6. Olano JP, Masters E, Hogrefe W, Walker DH. Human monocytotropic ehrlichiosis, Missouri. Emerg. Infect. Dis 2003;9:1579–1586. [PubMed: 14720399]
7. Roland WE, McDonald G, Caldwell CW, Everett ED. Ehrlichiosis--a cause of prolonged fever. Clin. Infect. Dis 1995;20:821–825. [PubMed: 7795080]
8. Olano JP, Hogrefe W, Seaton B, Walker DH. Clinical manifestations, epidemiology, and laboratory diagnosis of human monocytotropic ehrlichiosis in a commercial laboratory setting. Clin. Diagn. Lab. Immunol 2003;10:891–896. [PubMed: 12965923]
9. Stone JH, Dierberg K, Aram G, Dumler JS. Human monocytic ehrlichiosis. JAMA 2004;292:2263–2270. [PubMed: 15536115]
10. Morris, JA.; Harrison, LM. The microbiological investigation of sudden unexpected death in infancy. In: Kirkham, N.; Shepherd, NA., editors. Progress in Pathology. Vol. Vol. 7. New York: Cambridge University Press; 2007. p. 1-14.

Table 1

Necropsy summary for calves that expired after injection with *E. chaffeensis*.

	Calf No.		
Parameter (normal value range)	**A36**	**A33**	**A38**
Blood			
Phosphorus (4.3 – 7.8 mg/dL)	3.8	3.2	NA [a]
Potassium (4.0 – 5.8 mEq/L)	3.6	2.6	NA
Chloride (95.7 – 108.6 mEq/L)	95.0	102	NA
AST/SGOT [b] (45.3 – 110.2U/L)	517	229	NA
CK/CPK [c] (14.4–107.0 U/L)	4451	4879	NA
Glucose (42.1 – 74.5 mg/dL)	32	64	NA
Cerebrospinal fluid			
RBC (cells/ml)	NA	333	105
WBC (0–10 cells/ml)	NA	50	41
Protein (20–30 mg/dL)	NA	94	340
Serous fat atrophy	Pachymeninges	Spinal canal	Epidural space
Lungs (Gross lesions; Histopathology)	Pulmonary edema; acute pulmonary congestion	Pulmonary edema; no significant micropathology	Mucopurulent exudate; pyogranulomatous bronchopneumonia

[a] Not Available

[b] Aspartate aminotransferase

[c] Creatine kinase